Editorial introductions

Current Opinion in Rheumatology was launched in 1989. It is one of a successful series of review journals whose unique format is designed to provide a systematic and critical assessment of the literature as presented in the many primary journals. The field of Rheumatology is divided into 15 sections that are reviewed once a year. Each section is assigned a Section Editor, a leading authority in the area, who identifies the most important topics at that time. Here we are pleased to introduce the Journal's Section Editors for this issue.

SECTION EDITORS

Mariana J. Kaplan

Dr Mariana J. Kaplan is Senior Investigator and Chief of the Systemic Autoimmunity Branch at the National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, USA.

Dr Kaplan's research has focused on identifying mechanisms of organ damage and premature atherosclerosis in systemic autoimmunity. Her laboratory focuses on identifying how innate immunity promotes loss of tolerance and end-organ damage in SLE, rheumatoid arthritis and other systemic autoimmune diseases. Dr Kaplan also has an interest in identifying novel therapeutic targets to prevent premature vascular damage in systemic autoimmune diseases, using a bench to bedside approach. She has been principal investigator in various investigator-initiated clinical trials assessing new treatment approaches in autoimmunity. Dr Kaplan has served in numerous advisory and committee roles with the American College of Rheumatology, Rheumatology Research Foundation, American Association of Immunologists and Lupus Foundation of America and has been a member of the editorial boards of many immunology and rheumatology journals. She is a member of the American Society for Clinical Investigation and the Association of American Physicians and a recipient of the Henry Kunkel Young Investigator Award and the Edmund L. Dubois Memorial Lectureship, both from the American College of Rheumatology. She has also been recipient of the Evelyn Hess Award from the Lupus Foundation of America for her significant contributions to the understanding of systemic lupus erythematosus, and the Charles L. Christian award for her research impact in understanding lupus.

Polly J. Ferguson

Polly J. Ferguson received her BS and MD from the University of Iowa, USA. She completed her pediatric residency and pediatric rheumatology and immunology fellowship at the University of Virginia, USA, where she remained to complete a post-doctoral research fellowship. She is currently a Professor of Pediatrics and is the Director of Pediatric Rheumatology at the University of Iowa. Her NIH funded research program is focused on the etiology of autoinflammatory bone disorders including chronic recurrent multifocal osteomyelitis (CRMO). She has published more than 60 peer reviewed publications in addition to multiple book chapters and reviews.

Genetic advances in systemic lupus erythematosus: an update

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Purpose of review
More than 80 susceptibility loci are now reported to show robust genetic association with systemic lupus erythematosus (SLE). The differential functional effects of the risk alleles for the majority of these loci remain to be defined. Here, we review current SLE association findings and the recent progress in the annotation of noncoding regions of the human genome as well as the new technologies and statistical methods that can be applied to further the understanding of SLE genetics.

Recent findings
Genome-wide association studies (GWAS) have markedly expanded the catalogue of genetic signals contributing to SLE development; we can now explain more than 50% of the disease's heritability. Expression quantitative trait loci mapping with colocalization analysis of GWAS results help to identify the underlying causal genes. The Encyclopedia of DNA elements, Roadmap Epigenome, and the Blueprint Epigenome projects have jointly annotated more than 80% of the noncoding genome, providing a wealth of information (from healthy individuals) to define the functional elements within the risk loci. Technologies, such as next-generation sequencing, chromatin structure determination, and genome editing, will help elucidate the actual mechanisms that underpin SLE risk alleles.

Summary
Gene expression and epigenetic databases provide a valuable resource to interpret genetic association in SLE. Expansion of such resources to include disease status and multiple ancestries will further aid the exploration of the biology underlying the genetics.

Keywords
causal variants, epigenome, expression quantitative trait loci, genome-wide association studies, systemic lupus erythematosus

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease associated with a wide range of signs and symptoms varying among affected individuals and can involve many organs and systems, including the skin, joints, kidneys, lungs, central nervous system, and haematopoietic system. The population prevalence varies with ancestry, being more prevalent in non-European populations with a significant sex disparity towards women (6 : 1) during the years between menarche and menopause [1]. Although the exact cause of lupus is not fully understood, a strong genetic link has been identified through the application of family and large-scale genome-wide association studies (GWAS). The concordance rate in monozygotic twins (24%) is approximately 10 fold higher than in dizygotic twins (2%) [2,3]. A recent study from Taiwan reported that the heritability was 43.9% and the proportion of phenotypic variance explained by shared and nonshared environmental factors was 25.8 and 30.3%, respectively, suggesting nonheritable factors may play a considerable role in disease pathogenesis [4].

There are now more than 80 loci reported to be associated with the susceptibility of SLE. Here, we review current SLE association findings and the recent progress in the annotation of the noncoding region of the human genome as well as new

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KEY POINTS

- The discovery of SLE-associated risk variants has accelerated in the past 2 years with huge sample size genome-wide and meta-analysis studies revealing novel loci in both coding and noncoding regions of the genome.
- Expression quantitative trait loci mapping incorporating colocalization analysis of GWAS results help to identify the underlying causal genes.
- The Encyclopedia of DNA elements, Roadmap, and Blueprint projects which annotate noncoding regions have created comprehensive maps of the human genome.
- SLE-associated risk loci can be analysed bioinformatically, in the context of functional annotation to predict biological impact.
- Functional validation is required for designating variants as 'causal variants', and facilitated by the availability of genome editing tools such as clustered regulatory interspaced short palindromic repeats technology to artificially create the variant in a model system relevant for disease.

technologies and statistical methods, to apply this knowledge to the understanding of SLE genetics.

INSIGHTS FROM GENOME-WIDE ASSOCIATION STUDIES

Genetic linkage analysis and candidate gene association studies identified several SLE susceptibility loci (e.g., HLA-DR2/DR3) [5]. Nevertheless, the advent and application of GWAS dramatically advanced knowledge of the genetic cause of SLE.

There have been seven SLE GWAS in European population [6–10,11▪,12], six Asian GWAS [13–17,18▪], and one GWAS of Amerindian ancestry [19], as well as subsequent meta-analysis and large-scale replication studies [20,21,22▪,23], published since 2008. Currently, 84 genetic loci are implicated as SLE risk (Fig. 1: The CIRCOS plot [24] and Supplementary Table 1, http://links.lww.com/COR/A37), which, to avoid likely spurious associations, include genetic associations with a P value less than 5×10^{-8} tested in a total sample size of at least 1000 individuals. The interactive version of a continually updated resource with details on SLE associations can be access through the following link: http://insidegen.com/insidegen-LUPUS-Associations.html.

With the caveat that the majority of mechanisms remain to be elucidated, it appears that the risk loci associated with SLE influence immune cell function. Although functional studies are designed with a-priori hypotheses in mind, key pathogenic pathways that are likely influenced by SLE-associated gene products include: immune complex processing and phagocytosis; DNA degradation, apoptosis, and clearance of cellular debris; neutrophil and monocytes signalling; Toll-like receptor and/or type I interferon signalling; nuclear factor kappa beta (NF-κβ) activation; and B and T-cell function and signalling. Some genes associated with SLE may act through several pathways. For example, *TNFAIP3*, encoding the ubiquitin-editing enzyme A20, is a key regulator of NF-κβ-derived proinflammatory responses, which is involved in both adaptive and innate immune pathways [25,26]. These SLE susceptibility loci contain predominantly common (frequency of >0.1% in the general population) associated variants that have been confirmed among multiple ancestries, suggesting shared mechanisms in disease cause [27–29].

European genome-wide association studies

The largest European GWAS of SLE conducted by our group [11▪], comprised 7219 SLE cases and 15 991 controls of European decent, provided considerable power to detect disease risk loci. Notably, the study identified 43 susceptibility loci, 10 of which were novel loci: *SPRED2, IKZF2, IL12A, TCF7-SKP1, DHCR7-NADSYN1, SH2B3, RAD51B, CIITA-SOCS1, PLD2*, and *CXorf21*. One of the great challenges posed by interpreting GWAS data is determining the causal genes implicated by the genetic association data. As will be discussed below, we put some considerable effort into this process before naming the genes in the above list. Irrespective of the underlying causal genes, we can conclude that the heritability explained by the risk alleles mapped at these loci is 15.3%, which is a large increase over the 8.7% reported by So *et al.* [30] using the same measure.

Asian genome-wide association studies

An extensive large-scale fine mapping study using Immunochip conducted in 4478 SLE cases and 12 656 controls from six East Asian cohorts identified 10 novel loci [18▪] in Asians, encompassing *GTF2IRD1-GTF2I, DEF6, IL12B, TCF7, TERT, CD226, PCNXL3, RASGRP1, SYNGR1,* and *SIGLEC6*. Some of these were previously reported to be associated in Europeans, for example, *DEF6* and *TCF7*. The identification of these risk loci increased the explained heritability to 24% in Asian SLE. Nevertheless, the Immunochip was designed from predominant European genetic information and will, therefore, be less

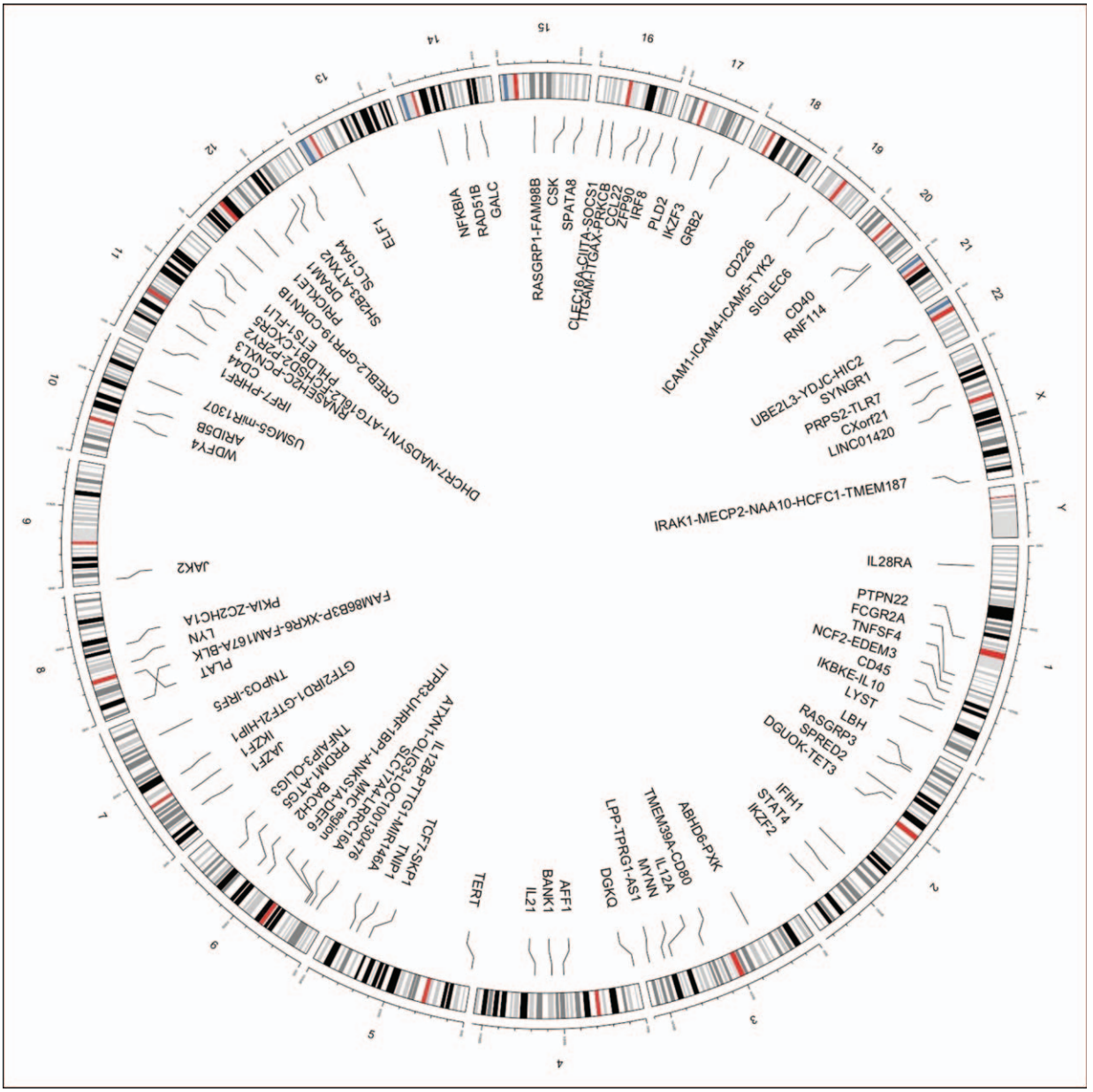

FIGURE 1. Systemic lupus erythematosus risk loci in genomic context. The CIRCOS plot [24] shows genes located within the systemic lupus erythematosus risk loci (84 in total) according to their genomic position. The full list of variants and locus genes for this plot is summarized in Supplementary Table 1, http://links.lww.com/COR/A37. The red block in each chromosome indicates the centromere of the chromosome. Each chromosome arm is divided into cytogenetic bands of hg19.

informative and not represented genetic variation in Asian population so well as in Europeans [31].

Trans ancestry meta analyses of genome-wide association studies

A comparison of genetic association signals across the genome in European and Asian populations suggested that SLE susceptibility loci were shared extensively between both populations [22▪]. This motivated a transancestral approach at the genome-wide level to provide evidence of shared genetic components in the two populations and search for additional SLE associated loci. The study by Morris *et al.* [22▪], that combined three GWAS from two ethnicities: Chinese (1659 cases and 3398 controls) and European (4044 cases and 6959 controls), found evidence of considerable commonality in terms of SLE association signals as well as mapping novel susceptibility loci, including *CD45, IKBKE, LBH, LPP-TPRG1-AS1, ATXN1, BACH2, GTF2I, JAK2, RNASEH2C*, and *ZFP90*. Notably, this

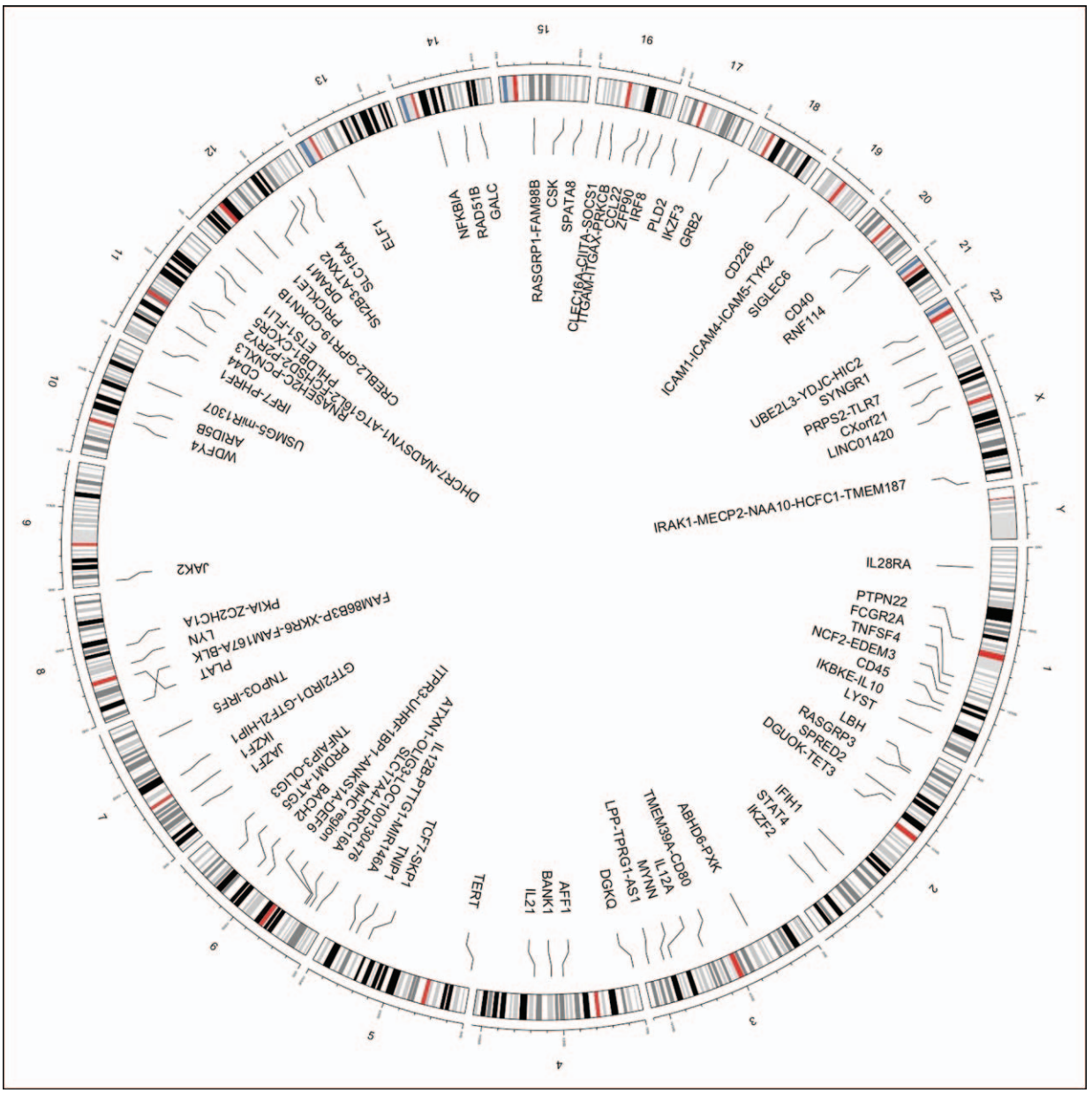

FIGURE 2. Box plots of genetic risk scores across the five major population groups. There are standard box plots showing medians, interquartile ranges, and whiskers, indicating 1.5 times the interquartile range (Tukey box plots) [21]. EUR, European, $N = 498$; AMR, Amerindian, $N = 347$; SAS, South Asian, $N = 487$; EAS, East Asian, $N = 503$; AFR, African, $N = 657$; from the 1000 genome phase 3 release. The dashed line represents the increase in prevalence with the rank order (R1 represents the lowest prevalence, and R4 the highest).

study suggested that the increased prevalence of SLE in non-European (including Asians) has a genetic basis by comparison of genetic risk scores between populations (Fig. 2) [22▪]. Moreover, by using all genotyped single nucleotide polymorphisms (SNPs) (DNA chip) to calculate heritability explained, the explained variation increase to 28% in Chinese patients and 27% Europeans using the GCTA algorithm [32]. Although there are still some uncertainties in the methodology for calculating heritability explained, this shows very strong evidence that we are making progress on the understanding of SLE heritability.

The latest large-scale transancestral study using Immunochip [33▪], comprising three ancestries: European (6748 cases and 11 516 controls), African-American (2970 case and 2452 controls), and Hispanic Amerindian (1872 cases and 2016 controls), have identified nine novel loci for European (*TMEM39A-TIMMDC1, DGKQ, LRRC16A, SLC17A4,*

OLIG3-LOC100130476, GTF2IRD1-GTF2I, FAM86B3P, PKIA-ZC2HC1A, and *GRB2*), two for African-American (*PTTG1-MIR146A* and *PLAT*) and two for Hispanic Amerindian (*GALC* and *CLEC16A*). By comparing results across different populations, both ancestry-dependent and ancestry-independent contributions to SLE risk are identified with the caveat of unequal cohort sizes. The study reveals evidence of sharing of genetic risk loci between ancestries as well as evidence that each individual population carries unique genetic risk factors at the locus level and at the allelic level.

Missing heritability

In summary, the chip heritability identified by the latest GWAS have explained around 28% of the disease heritability: a marked improvement on 8.3% calculated in 2011 [30]. Although the overall heritability of complex disease is complicated to estimate, a study in European population from a family survey did estimate a heritability of $66 \pm 11\%$ for SLE [34], indicating there are more than 50% of missing heritability in SLE from current GWASs. If we assume that the total heritability is 43.9% (with 25.8% for shared environmental factors) estimated from a Taiwanese population [4], there is still one-third of heritability left to explain. Explanations for the missing heritability, including larger numbers of variants of smaller effect, rarer variants (possibly with larger effects) that are not present on genotyping arrays or structural variants poorly captured by existing arrays, as well as epigenetic modifications, have been suggested [35]. Innovations in genotyping and sequencing technologies, like the Immunochip platform [18▪,33▪] and next-generation sequencing (NGS, as described below) will advance the investigation into common and rare variants and potential effects on the immune system, enhancing our understanding of the genetic risk of SLE.

The linkage disequilibrium that exists in the human genome facilitates the mapping of risk loci by reducing the number of genetic variants required for GWAS; however, the same correlation between genetic polymorphisms at these susceptibility loci then bedevils attempts to identify the actual causal allele(s) at risk loci. Bayesian fine mapping approaches had been proposed to derive smaller sets of SNPs (termed 'credibility sets') as the most likely causal variants at risk loci [36]. Nevertheless, statistical methods are inadequate to fully resolve the problem caused by linkage disequilibrium. To further pursue likely causal SNPs within any given credibility set, the functional effect of SNPs can be studied *in silico*. As the majority of variants within causal credibility sets are noncoding [37,38], function is inferred using gene transcript expression data and epigenetic modification data (as described below) (Figs. 3 and 4).

APPLICATION OF EXPRESSION QUANTITATIVE TRAIT LOCI MAPPING TO GENOME-WIDE ASSOCIATION STUDIES RESULTS

Assisted by dense genome coverage of the reference panel from the 1000 Genome project [39], imputation and Bayesian inference provided evidence for missense variants underpinning association for eight genes, including *PTPN22, FCGR2A, NCF2, IFIH1, WDFY4, ITGAM, PLD2*, and *TYK2* [11▪]. However, as mentioned above, the majority (85–90%) of disease associated loci in SLE are located outside of protein-coding regions, and so might exert their function through altering gene expression rather than by altering protein structure. Of note, an over-representation ($n = 16$) of transcription factors among the 43 SLE susceptibility genes have been annotated in our recent European GWAS [11▪], further indicating that perturbed gene regulation was a major functional risk factor for SLE. Expression quantitative trait loci (eQTL) mapping, which combines genome-wide expression profiling and genome-wide marker-based genotyping, takes advantage of the heritability of gene expression profiles to identify genetic variants that correlate with changes in gene expression. eQTL can be classified as 'in *cis*' (locally) or 'in *trans*' (at a distance) based on their physical distance from the regulated gene.

Some studies [18▪,23] used public databases, such as the whole blood eQTL browser (http://genenetwork.nl/bloodeqtlbrowser/) [40] and tissue-specific Genotype-Tissue Expression (GTEx) portal (http://www.gtexportal.org/home/) [41], to determine whether the disease-associated SNP is a significant eQTL. Of note, limitations exist when applying eQTL analysis to the GTEx whole blood data sets; for example, we seek eQTL in specific immune cell subsets when studying autoimmune disease. To highlight the potential causal genes at the susceptibility loci robustly, it is essential to integrate the disease association and eQTL data using a colocalization approach. That is, to establish that the same genetic variants that underlie the disease association also underlie the eQTL. The presence of linkage disequilibrium in the genome can readily obfuscate this overlap. Colocalization methods, like the regulatory trait concordance (RTC) [42], conditional analysis [32], and Bayesian colocalization [43], can be employed to infer that the disease association and

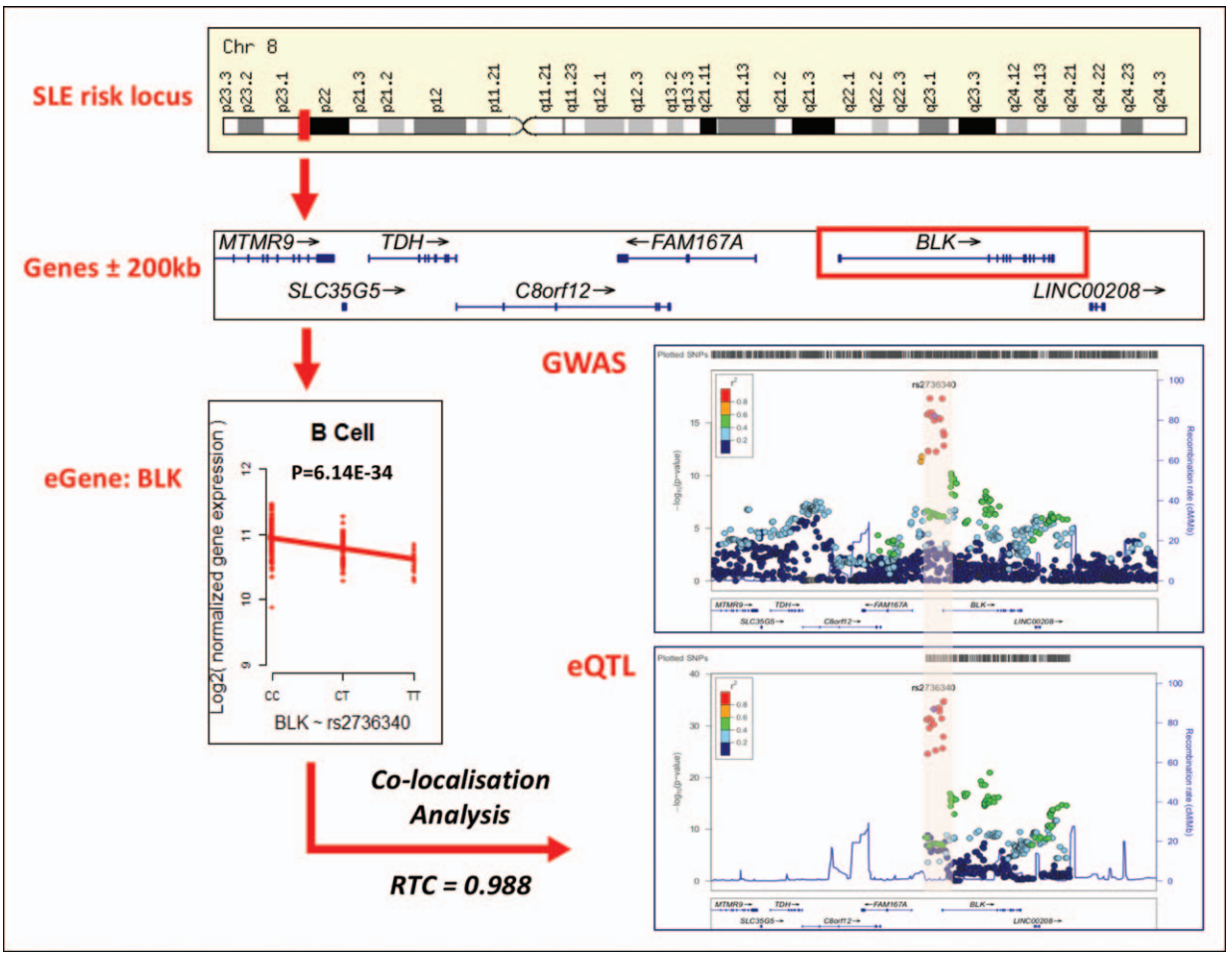

FIGURE 3. Overview of colocalization analysis of genome-wide association studies and expression quantitative trait loci. This figure shows an example of expression quantitative trait loci analysis and the application of regulatory trait concordance for the causality inference. Firstly, we subset the genes within the *cis*-window (±1 Mb) of the disease-associated locus (rs2736340) and perform linear regression against the genotypes of the SNP. Colocalization analysis of the genome-wide association studies signal and the expression quantitative trait loci signal was performed by calculating the regulatory trait concordance score. SNP-expression pairs with regulatory trait concordance more than 0.9 were considered causal.

eQTL have the same allelic basis. As many variants have weak eQTL effects, erroneous conclusions will be made if analyses for colocalization are not performed. An example of colocalization analysis of eQTL and GWAS is shown in Fig. 3.

Recent studies by Morris *et al.* [11▪,22▪] and Odhams *et al.* [44▪] examined the functional outcome of SLE associated variants through the integration of GWAS and eQTL data from various cell types *ex vivo*, involving T cells, B cells, natural killer (NK) cells, stimulated and resting monocytes, as well as lymphoblastoid cell lines (LCL). By integrating the results of eQTL and RTC analysis, they found evidence to support the role of causal genes as candidates at a given locus. For example, a SLE risk variant rs9652601 is located within *CLEC16A* (C-Type Lectin Domain Family 16 Member A) – a gene previously reported as relating to other autoimmune diseases [45]. However, eQTL and RTC analyses suggest that *SOCS1* (Suppressor of Cytokine Signalling 1) is more likely be a causal gene at the locus (eQTL FDR $\leq$ 0.01 & RTC score $\geq$0.9). Moreover, the Odhams *et al.*'s study [44▪] illustrated the benefits of using RNA-seq as opposed to microarrays for eQTL mapping, due to more informative data generated by RNA-seq. With RNA-seq, transcript profiling can be done on the gene-level, exon-level, and splice-junction-level, which is more effective in explaining potential regulatory mechanisms.

Nevertheless, we believe that many eQTLs related to SLE risk alleles remain unidentified, data from diverse stimulations and time points will be required, as well as gene expression data from patient material, to reveal the full eQTL landscape of SLE genetics.

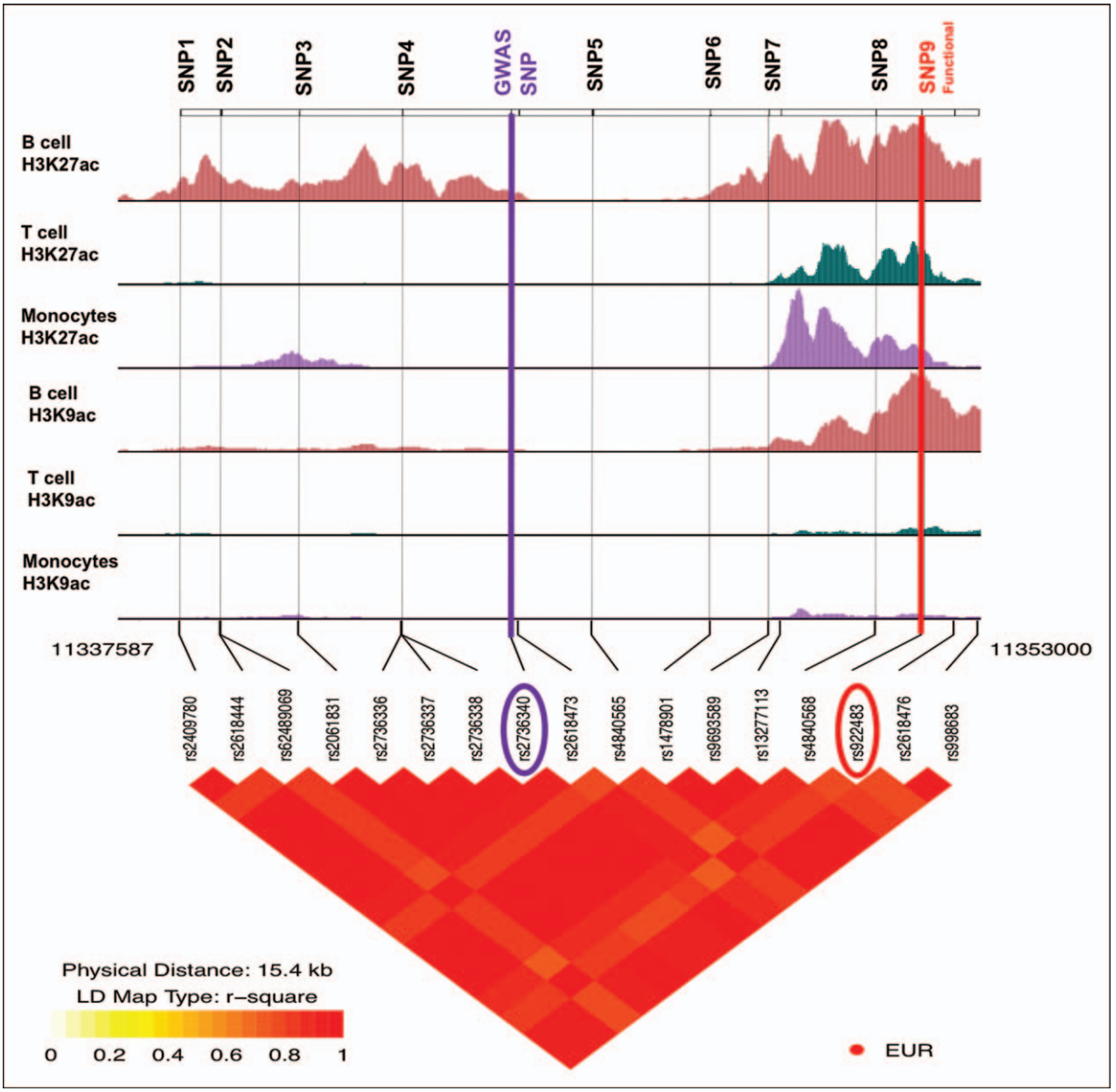

FIGURE 4. Schematic overview of fine mapping causal SNPs by integrating genetics and epigenetics. This figure illustrates the functional annotation approach by an example, *BLK* (data unpublished). The epigenetic data of two histone markers (H3K27ac and H3K9ac) from three primary cell types (B cell, T cell, and monocytes) (Roadmap Project) are represented for the target locus. This region contains 17 SNPs derived from 99% Bayesian credibility set of the risk locus. Rs2736340 is associated with systemic lupus erythematosus (Fig. 3). rs922483 overlaps H3K27ac in all three cell types while it overlaps the H3K9ac peak in B cells only. Furthermore, rs922483 is in strong linkage disequilibrium ($r^2 = 0.98$) with rs2736340, indicating that there is transitive evidence due to the linkage disequilibrium that rs922483 is also associated with systemic lupus erythematosus and is an expression quantitative trait loci. Therefore, rs922483 is the most likely functional SNP in this risk locus.

EPIGENETICS TO ANNOTATE FUNCTIONAL/REGULATORY VARIANTS

An approach that is complementary to eQTL analyses to examine the regulatory function of non-coding genetic variants is to study gene regulation with epigenetics. Epigenetic modifications, a term coined to describe genome-wide chromatin modification, including DNA methylation, histone modifications, chromatin accessibility, microRNA regulations, and two-dimensional chromatin interactions [46], constitute an additional layer of genomic regulation and may serve as a dynamic link between genotype and phenotype. Such changes in DNA and chromatin structure correlate with changes in chromatin accessibility and transcription factor binding.

The Encyclopedia of DNA elements (ENCODE) project (https://www.encodeproject.org/) [47] has systematically mapped regions of transcription, transcription factor association, chromatin structure and histone modification, and assigns biochemical functions for 80% of the genome, in particular outside of the protein-coding regions. Overall, the project has provided an expansive resource to define the functional DNA elements for biomedical research, whereas the available cell types or cell lines are limited. The cells of closest immune relevance in ENCODE Tier 1 and Tier 2 are LCLs (GM12878), B cells (CD20+), and monocytes (CD14+), as well as T cells (CD4+) and peripheral blood mononuclear cell in Tier 3. A recent Immunochip study in Asians [18▪] took advantage of ENCODE data to map the underlying loci. For example, one of the signals (rs73366469) identified in this study was located between two 'general transcription factor' genes, *GTF2I* and *GTF2IRD1*. By integrating the ENCODE data, they found that an indel SNP rs587608058 ($r^2 = 0.81$), ~1000 bp from rs73366469, lay within conserved enhancer, active chromatin and transcription factor binding sites in LCLs and CD4+ T cells. In addition, this region was found to overlap the transcription start sites for *GTF2I* and *VCF* through chromatin interacting analysis and chromosome confirmation capture (Hi-C) analysis, providing evidence for the potential causal variants and genes at this locus for further study.

The Roadmap epigenomics project (http://www.roadmapepigenomics.org/) [48▪▪] integrated analysis of 111 reference human epigenomes to obtain a comprehensive map of the human epigenomic landscape across a large collection of primary cells (including immune cells) and tissues. This map is extremely useful for studies of genome interpretation, gene regulation, cellular differentiation, genome evolution, genetic variation, and human disease. In our meta GWAS analysis of Chinese and European data [22▪], the histone modification markers, including acetylation markers (H3K27ac and H3K9ac) and methylation markers (H3K27me3 and H3K9me3), from blood cell types were used to investigate the potential regulatory function of the target risk loci. For example, there are several genes, including *SRGAP2, SRGAP2D, IKBKE, RASSF5, EIF2D,* and *DYRK3*, located within ±200 kb of the lead GWAS SNP rs2297550. The GWAS SNP was also found to be a putative eQTL for *IKBKE*, with the SLE risk allele correlated with reduced expression in CD4+ T cells [49], CD19+ B cells [50], and NK cells (data unpublished), but with increased expression in CD14+ monocytes [51]. *IKBKE* encodes a noncanonical I-kappa-B kinase that is essential in regulating inflammatory responses to viral infection by activating the type I interferon, NF-kB and signal transducer and activator of trnascription signalling pathways, suggesting *IKBKE* might be the potential causal gene. Moreover, there is an intense histone acetylation peak around the associated SNP rs2297550, indicating that rs2297550 may be a potential causal variant [22▪]. Figure 4 shows an example of fine mapping causal SNPs by integrating genetics and epigenetics.

Another recent completed large-scale epigenomic project, the Blueprint project (http://www.blueprint-epigenome.eu/) [52,53▪▪,54▪▪], has impressively shown how epigenetic information and analyses can help to study the cellular mechanisms associated with complex human diseases. Moreover, the Blueprint consortium generated three comprehensive reference panels, including genome (whole genome sequencing), transcriptome (RNA-seq), and epigenome (DNA methylation and histone modification), in three immune cells (Neutrophils, monocytes and T cells) from nearly 200 individuals to characterize the contributions of diverse genomic inputs to transcriptional variation. Summary data from these panels can be accessed through http://blueprint-dev.bioinfo.cnio.es/WP10/.

High-resolution maps of promoter interactions [53▪▪] generated by 'Promoter capture Hi-C' (PCHi-C) make it possible to study the long-range regulatory in the three-dimensional nuclear space. By integrating PCHi-C data with disease-associated SNPs generated by GWAS, we can prioritize the putative target genes for the risk loci. The promoter interactomes map may serve as a more robust method to define *cis*-eQTL rather than by distance, revealing insights into genomic regulatory mechanisms of diseases.

NEXT-GENERATION SEQUENCING IN THE GENOME RESEARCH

With the development of NGS, high-throughput technologies that are now widely used in genome research, any part of the genome can be sequenced. Based on the coverage of the genome, NGS strategies can be classified by scale: target region sequencing, whole-exome sequencing (WES), and whole-genome sequencing (WGS). Targeted resequencing of risk loci in disease cohorts may facilitate the identification of rare variants at common-allele-associated loci [55]. WES captures all coding exons covering 1–2% of the genome. Nevertheless, as mentioned above, approximately 85–90% of the risk loci associated with SLE are located outside the coding-regions. Compared with WES, WGS can capture the majority of the genome, which

facilitates delineation of exon duplications and gene fusions and noncoding regions that might be missing by WES. WGS performs better than WES in the identification of copy number variation and structural variation in the genome which facilitates the identification of coding variants in more complex areas of the genome. However, the higher cost and time consuming bioinformatics analyses that are required to process the data, restrict the application of WGS [56]. In future, with the decreasing cost of sequencing and newly developed computation algorithms, WGS will be increasingly utilized.

Incorporating with a wide range of chromatin profiling experiments, NGS is applied to investigate chromatin biology by identifying genomic loci that are occupied by nucleosomes, bound to transcription factors, or accessible to nuclease cleavage [57]. Technologies such as ChIP-seq [58], FAIRE-seq, DNase-seq [59,60], Hi-C [61], and ATAC-seq [62] enable genome-wide investigations of a broad range of chromatin phenomena in both qualitative and quantitative ways. Moreover, when introducing NGS to the transcriptome level (RNA-seq), it can be used to detect changes in gene expression, as discussed earlier in this review [40,63,64].

CONCLUSION

Linkage analysis and GWAS studies fail to fully explain disease heritability and do not address the causal nature of risk variants. NGS continues to fuel the discovery of disease-associated common and rare variants. The advances in analysis tools, such as Bayesian fine mapping approaches and high performance computation algorithms, help to make full use of the current massive data to uncover relationships and infer the causality among complex data. Comprehensive sets of functional annotations (ENCODE, Roadmap, and Blueprint projects) in the context of complex genomic structure can be used to predict function and guide experimentation, such as precision genome editing with the CRISPR-Cas (clustered regularly interspaced short palindromic repeats/CRISPR-associated) [65,66], to address the long standing question of disease mechanism and heterogeneity. Nonetheless, we still have not yet fully exploited analysis of GWAS data, such as genetic studies in non-European populations with different linkage disequilibrium, especially important in SLE given the prevalence; eQTL and epigenetic data in cells from non-European populations for functional annotation; epigenetic data in larger cohorts to look at interindividual variation; eQTL and epigenetic data from disease cohorts, to look for disease specific effects [67▪▪]. Studies based on these cohorts will advance our understanding of the disease mechanism, and ultimately speed up the arrival of the era of personalized medicine with genomic data incorporated into diagnosis, prognosis, and treatment in clinics.

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Conflicts of interest

There are no conflicts of interest.

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- ▪ of special interest
- ▪▪ of outstanding interest

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An update on lupus animal models

Wei Li, Anton A. Titov, and Laurence Morel

Purpose of review
The complexity and heterogeneity of the clinical presentation in systemic lupus of erythematosus (SLE), combined to the inherent limitations of clinical research, have made it difficult to investigate the cause of this disease directly in patients. Various mouse models have been developed to dissect the cellular and genetic mechanisms of SLE, as well as to identify therapeutic targets and to screen treatments. The purpose of this review is to summarize the major spontaneous and induced mouse models of SLE and to provide an update on the major advances they have contributed to the field.

Recent findings
Mouse models of SLE have continued to contribute to understand the cellular, signaling and metabolic mechanisms contributing to the disease and how targeting these pathways can provide therapeutic targets. Whenever possible, we discuss the advantage of using one model over the others to test a specific hypothesis.

Summary
Spontaneous and induced models of lupus models are useful tools for the study of the cause of the disease, identify therapeutic targets and screen treatments in preclinical studies. Each model shares specific subsets of attributes with the disease observed in humans, which provides investigators a tool to tailor to their specific needs.

Keywords
B cells, mouse models, systemic lupus of erythematosus, T cells, therapeutic targets

INTRODUCTION

Systemic lupus of erythematosus (SLE) is a chronic disorder that is characterized by the over-production of antinuclear autoantibodies (ANA) resulting in the formation of immune complexes that induce tissue inflammation and destruction in multiple organs, including the kidneys [1]. The exact cause of SLE is still unknown, but there is a strong evidence that a combination of environmental exposures, genetic predisposition, cellular dysfunctions and hormonal factors lead to the development of SLE [2]. Given the high degree of clinical heterogeneity in SLE patients, preclinical mouse models summarized below (Table 1) have been very valuable to investigate the cause of SLE as well as to identify and validate therapeutic targets.

These mouse models of SLE are either spontaneous or induced, but none of them fully represents the entire clinical spectrum found in SLE patients. However, each model presents an overlapping subset of human lupus phenotypes and offers specific features of interest to address-specific preclinical purposes. In addition to polygenic models, a number of mouse models are based on a single gene knock-out or transgenic expression of genes which result in lupus-like phenotypes [11]. These strains have been instrumental in delineating functional pathways in SLE as well as the involvement of specific genes in maintaining systemic immune tolerance and preventing immune complex-induced inflammation. There have been numerous reviews of mouse models of SLE starting from the foundational work published over 30 years ago [12] that has been followed by many updates. Many reviews have focused on specific aspects of these models, such as the genetic links between human and mouse SLE [11], or the mechanisms leading to systemic autoimmunity and clinical lupus in these models [13]. The present review will briefly summarize the most common mouse of SLE,

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KEY POINTS

- Spontaneous and induced models of lupus models are useful tools for the study of the cause and mechanisms of the disease.
- Mouse models of lupus have advanced the field through the identification therapeutic targets and the evaluation of corresponding treatments in preclinical studies.
- Each model shares specific subsets of attributes with the disease observed in humans, which provides investigators a tool to tailor to their specific needs.

stressing their unique features. We will then provide an update on the major advances they have contributed to the field, and whenever possible, we will discuss the advantage of using one model over the others to test a specific hypothesis.

SPONTANEOUS MOUSE MODELS OF SYSTEMIC LUPUS OF ERYTHEMATOSUS

NZB/W F1

In 1960s, the NZB/W F1 model of lupus referred to the F1 hybrid between the NZB and NZW strains [3]. NZB mice show limited hemolytic autoimmune anemia, whereas NZW mice are nonautoimmune. However, their F1 hybrids develop severe lupus-like phenotypes, including a strong female bias, splenomegaly, elevated serum ANA mostly directed against DNA. Immune complex-mediated nephritis develops by 5–6 months of age, leading to renal failure and death at 10–12 months of age [12]. Overall, NZB/W F1 is a classic model used to study the genetic underpinning of SLE [11] as well as drug responses in many preclinical studies, including the inhibition of B cell activating factor (BAFF) [14], the role of type 1 interferon [15] and the identification of biomarkers of lupus nephritis [16].

New Zealand Mixed

An accidental backcross between NZB/W F1 and NZW followed by brother–sister mating generated 27 different recombinant inbred strains of New Zealand Mixed (NZM) mice among which NZM2328 and NZM2410 are now used as lupus models [4–6]. The clinical manifestations in NZM strains are similar to that of NZB/W F1 mice, whereas there are some differences in renal disorder [16,17] and the response to BAFF inhibition [18]. The main advantage of the NZM strains over NZB/W F1 is that they have homozygous genomes, which has facilitated genetic analyses [11]. From the NZM2410 strain, a novel congenic model has been produced that combines the three susceptibility loci, *Sle1*, *Sle2* and *Sle3*, that are necessary and sufficient to induce a lupus phenotype on a nonautoimmune C57BL/6 (B6) genetic background [19]. The B6.NZM2410.*Sle1*.*Sle2*.*Sle3* has the unique advantage to share 95% of its genome with B6, providing a robust control for immunological and genetic studies. The corresponding single (mostly *Sle1*) and bicongenic (*Sle1*.*Sle3*) are

Table 1. Classical mouse models of lupus

Mouse model	Generation/protocol	Sex bias	Main clinical manifestations
Spontaneous models			
NZB/W F1 [3]	F1 hybrid between NZB and NZW strains	Female	Lymphadenopathy, splenomegaly, anti-dsDNA IgG, IC-mediated GN
NZM2410/2328 [4–6]	Backcross between NZB/W F1 and NZW followed by brother–sister mating	Female	Overlaps with NZB/W F1
MRL/*lpr* [7]	*lpr* mutation in *Fas* gene on MRL background	Both	Lymphadenopathy due to accumulation of DN B220$^+$ T cells, DNA and RNA-directed autoantibodies, IC-mediated GN and dermatitis
BXSB/*Yaa* [8]	Backcross of (B6 X SB/Le) F1 to SB/Le	Male	Lymphadenopathy, anti-DNA, RNA and gp70 autoAbs, monocytosis, IC mediated GN
Induced models			
Pristane-induced lupus [9]	*i.p.* injection of pristine	Female	Type I interferon mediated, autoAb, GN, arthritis, anemia, serositis (strain dependent)
cGVHD [10]	(1) DBA → BDF1 (injection of spleen cells)	Female	AutoAb, GN, polyclonal B-cell and T-cell activation, proteinuria (CD8+ T cell dependent)
	(2) B6 ↔ B6.Bm12 (injection of spleen cells)	Female	AutoAb, GN, polyclonal B-cell and T-cell activation, proteinuria (donor CD4+ T-cell dependent)

cGVHD, chronic graft-versus-host disease; IC, immune complexes.

well suited to breed to B6-based gene knockouts. For instance, deletion of the plasmacytoid dendritic cells (pDC)-specific transcription factor *Tcf4* in B6.*Sle1*.*Sle3* mice provided genetic evidence that pDCs are critically involved in the development of SLE [20▪].

MRL/*lpr*

The MRL strain was developed by crossing several stains, including LG/J, C3H/Di, C57BL/6 and AKR/J [12]. One of the MRL substrains carrying a spontaneous mutation named *lpr* for lymphoproliferation developed an SLE-like phenotype characterized by accumulation of double negative ($CD4^-CD8^-$) $B220^+$ T cells. Double negative T cells are autoreactive [21] and expanded in SLE patients [22], making this model specifically relevant to SLE pathogenesis. *Lpr* corresponds to nonfunctional transcripts of the *Fas* gene, a major regulator of apoptosis in immune cells [23]. Both male and female MRL/*lpr* mice are affected and produce autoantibodies against dsDNA and Sm, leading to large amounts of immune complex that induce renal and skin disorder [7]. MRL/*lpr* mice develop a massive lymphadenopathy that is not observed in SLE patients. However, in addition to expanded double negative T cells, this model has the advantage of a rapid and severe disease development as compared with the other spontaneous models. Notably, the MRL/*lpr* strain has been used to dissect the role of toll-like receptor 7 and TLR9 in lupus [24], to compare TLR activation in B cells and dendritic cells [25] and to dissect the development of extrafollicular autoreactive B cells [26▪]. In addition, B6.*lpr* mice, which develop systemic autoimmunity without clinical disorder and a reduced lymphadenopathy, have been used to investigate various pathways, including the involvement of Th17 T cells in lupus [27].

BXSB/*Yaa*

A recombinant inbred strain derived from the backcross of (B6 X SB/Le) F1 to SB/Le, termed BXSB/Mp (BXSB/*Yaa*), develops a lupus-like disease with lymphoid hyperplasia, immune complex-mediated nephritis, ANA and high-serum retroviral glycoprotein gp70 titers [7,28]. Nephritis leads to the death of BXSB/*Yaa* males in about 5 months and BXSB females in 14 months. The rapid-onset disease in males is attributable to the Y-autoimmune accelerator (*Yaa*) locus, which is due to a translocation from the X to the Y chromosome, duplicates 16 genes, including TLR7 [29,30]. TLR7 regulates the activation of the type 1 interferon pathway by RNA complexes, a critical pathway in SLE pathogenesis [31]. Therefore, in spite of its presentation in males, the BXSB/*Yaa* strain is uniquely suited to model the consequences of an overeactive TLR7/Type 1 interferon pathway.

INDUCED MOUSE MODELS OF SYSTEMIC LUPUS OF ERYTHEMATOSUS

Pristane-induced lupus

Pristane is an isoprenoid alkane found at high concentration in mineral oil. Intraperitoneal injection of pristane is a standard method to obtain ascitic fluid enriched in mAbs. Antiribonucleoprotein, anti-DNA and antihistone autoantibodies are found in BALB/c mice after pristane injections. Pristane-treated mice also have immune complex deposition in the kidney causing severe nephritis [32]. Strain differences in the response to pristane have been observed [33], illustrating the role of gene/environment interactions in lupus susceptibility. Pristane-induced lupus is more severe in females than in males, at least in the SJL strain [34]. Pristane-induced lupus is driven by a strong type 1 interferon response [35], and this model is, therefore, well suited to investigate the type 1 interferon signature present in many SLE patients, but much weaker in spontaneous mouse models of this disease. This model is also useful to test the impact of a specific gene on lupus development directly in a nonautoimmune strain, such the protective effect of TLR9 evaluated in BALB/c.$Tlr9^{-/-}$ mice treated with pristane [36▪].

Chronic graft-versus-host disease models

Induced chronic graft-versus-host disease (cGVHD) models require injections of donor lymphocytes into a semiallogenic recipient to induce a lupus-like syndrome. In the parent → F1 model, DBA/2 strain spleen cells are injected into (C57BL/6 X DBA/2) F1 (BDF1) recipients, whereas in the other, B6 spleen cells are injected into class II major histocompatibility complex-missmatched B6.bm12 recipients or reversely. In both models, donor $CD4^+$ T cells react to host B cells triggering the polyclonal activation of autoreactive B cells, and eventually, lupus-like syndrome [10]. Compared with the other models, cGVHD is easy to control, adjustable to investigator's needs and generally presents with a reduced interindividual variability. In addition, autoimmune and clinical manifestations of SLE develop relatively rapidly over a period of weeks, instead of months for the other models. Finally, because the activation and expansion of donor T cells play an essential role in cGVHD response, it is easy to track them relative to host cells by flow cytometry.

These models also allow the study of the effect of treatments or genetic modifications in donor cells to alter the course of the cGVHD response. The bm12 model is particularly useful to test the effect of single genes or alleles on the development of systemic autoimmunity on a B6 genetic background. This approach has been used to evaluate *Slamf6* isoforms as lupus susceptibility alleles for the *Sle1b* locus [37,38], and to identify the association of a naturally occurring polymorphism in the *G-CSF* gene with resistance to autoimmune activation [39,40].

RECENT INVESTIGATIONS OF THERAPEUTIC TARGETS WITH MOUSE MODELS OF LUPUS

Table 2 lists recent treatments or genetic approaches that have been used in mouse models of lupus.

T-cell targets

Cellular metabolism has been identified as a major checkpoint of $CD4^+$ T-cell effector functions [67]. Consequently, manipulating T-cell metabolism may be a promising avenue to treat immune-related diseases [68]. In lupus mice as well as SLE patients, $CD4^+$ T cells have an elevated metabolism. Treatment with a combination of metformin and glucose inhibitor 2-deoxyglucose normalized T-cell metabolism and reversed disease in several mouse models of SLE [41▪▪,42▪]. Natural compounds isogarcinol and quercitrin ameliorated disease in a cGVHD mouse model by decreasing $CD4^+$ T-cell activation as well autoantibody production [43,44]. Quercitrin is a derivative of quercetin, a glycolytic inhibitor, suggesting that metabolic inhibition was a mechanism responsible for the therapeutic effect.

Table 2. Treatments tested in mouse models of systemic lupus of erythematosus

Gene target	Cell target	Model	Treatment	Main manifestations	Reference
T-cell targets					
Cellular metabolism	CD4 T cells	B6.Sle1.Sle2.Sle3 BWF1 B6.*lpr*	Metformin, 2-deoxyglucose	AutoAb↓, GN↓ Immune activation↓	[41▪▪,42▪]
Cellular metabolism	CD4 T cells	cGVHD	Isogarcinol	Proteinuria↓, autoAb↓, GN↓	[43]
Cellular metabolism	CD4 T cells	cGVHD	Quercitrin	Proteinuria↓, autoAb↓, GN↓	[44]
B7-1	T-cell-APC interaction	Pristane-induced	B7-1 shRNA and anti-B7-1 mAb	ANA↓, anti-dsDNA IgG↓	[45,46]
ICOS-B7RP-1	Tfh	BWF1	Anti-ICOS-B7RP-1	Proteinuria↓, anti-dsDNA IgG↓	[47]
ICOS-B7RP-1	Tfh	MRL/*lpr*	ablation of ICOS ligand in $CD11c^+$ cells	Kidney/lung inflammation↓	[48▪▪]
IL-21	Tfh	B6.Sle1.Yaa	Anti-IL-21 MAb	GC B cells↓, CD138hi↓ IgG2c↓, autoantibodies↓	[49]
IL-21	Tfh	cGVHD	IL-21	Host B cell↓, autoantibody↓, renal disease↓	[50▪]
IL-21	Tfh	MRL/*lpr*, BWF1, BXSB	IL-21R Fc	IgG↓, proteinuria↓, antidsDNA↓	[51,52▪,53]
B-cell targets					
BAFF	B cells	MRL/*lpr*	BAFF-R Fc	Tertiary lymphoid structures and nephritis↓	[54]
BAFF	B cells	NZM2328	KO BCR3 with TACI or BCMA	BAFF-BCMA and/or BAFF-TACI combinations contribute to SLE	[55]
BTK	B cells	BWF1,MRL/*lpr*, pristane-induced, BXSB	Various Btk inhibition	GN↓, ANA↓, IC↓	[56–59]
miR-148	B cells	MRL/*lpr*	Increased miR148	GN↓	[60▪▪]
miR-155	B cells	B6.*lpr*	miR155 KO	ANA↓ B-cell signaling↓	[61]
Proteasome	Plasma cells	BWF1,MRL/*lpr*	Proteasome inhibitor	ANA↓, GN↓, Survival↑	[62]
Other targets					
NLRP3	Macrophages	BWF1	NLRP3 inhibitor	ROS/ NAPDH/COX-2↓, GN↓	[63]
NLRP3	Macrophages	Pristane-induced	NLRP3 gain-function	Proteinuria↑ and GN↑	[64]
IRAK4	TLR pathway	BWF1, MRL/*lpr*	IRAK4 inhibitor	Proteinuria↓, dsDNA↓, GN↓	[65]
Topoisomerase I	dsDNA binding	MRL/*lpr*	Topoisomerase I inhibitor	Nephritis and skin lesions↓	[66]

ANA, antinuclear autoantibodies; B7RP-1, B7-related protein-1; BAFF, B cell activating factor; COX, cyclooxygenase-2; IC, immune complexes; ICOS, Inducible T-cell COStimulator; IRAK4, IL-1R-associated kinase 4; ROS, reactive oxygen species; Tfh, T follicular help cells.

The interactions between B7-1 and 2 on the B cell/antigen presenting cell side and CD28/cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) on the T cell side are cardinal regulatory pathway of the immune response, and there have been numerous attempts to target them therapeutically [69]. Based on studies in mouse models, CTLA-4-Ig (abatacept) is now in clinical trial for the treatment of lupus nephritis [70]. In the pristane-induced lupus model, the specific blockade of the interaction between B7-1 and CD28 decreased serum ANA and antidsDNA IgG [45].

T follicular help cells (Tfh) are a $CD4^+$ helper T-cell subset specialized for provision of help of B cells which plays an essential role in germinal center formation, affinity maturation and the development of most high-affinity antibodies [71]. Tfh cells are expanded in mouse models of lupus, and the level of circulating Tfh cells correlates with disease severity in SLE patients [72]. Consequently, therapeutic targeting of Tfh cells has been proposed for SLE patients and lupus mouse models through the IL-21, Inducible T-cell COStimulator (ICOS) and OX40 pathways. Genetic approaches or a soluble IL21R-Fc protein have demonstrated that blocking the IL-21 pathway prevented or greatly ameliorated disease in several mouse strains [52▪,73]. A recent preclinical study showed that treatment of B6.Sle1.*Yaa* mice with an anti-IL-21 antibody reduced germinal center B cells, $CD138^{hi}$ plasmablasts, IFN-γ-dependent IgG2c production and autoantibodies, indicating that Tfh cell-derived IL-21 is critical for pathological B cell cues in lupus [49]. However, targeting the IL-21 pathway may have unintended consequences in $CD8^+$ T cells. In BXSB.Yaa, IL-21 signaling is essential for the maintenance of $CD8^+$ suppressor T cells [74]. Moreover, in the parent → F1 cGVHD model, treatment with IL-21 strongly promoted donor $CD8^+$ T-cell expansion and rescued defective donor antihost CTLs, resulting in host B-cell elimination, decreased autoantibody levels and attenuated renal disease, despite evidence of concurrently enhanced $CD4^+$ T cell help for B cells [50▪]. Another approach to eliminate Tfh cells has been to target ICOS/B7RP-1 interactions. Treatment of NZB/W F1 mice with an anti-B7RP-1 antibody decreased the number of Tfh cells and germinal center B cells and ameliorated disease manifestations [47]. It is also been reported that the selective ablation of ICOS ligand in $CD11c^+$cells, but not in B cells, dramatically ameliorated kidney and lung inflammation in MRL/*lpr* mice [48▪▪].

B-cell targets

BAFF is a cytokine that is required for B-cell development and survival. Largely based on studies in mouse models [75], BAFF blockade has been the first and only biologic treatment approved to treat lupus. BAFF also plays a previously unappreciated role in lupus nephritis by inducing renal tertiary lymphoid structures and regulating the position of T cells in the glomeruli of MRL/*lpr* mice [54]. Moreover, genetic approaches in the NZM2328 mice demonstrated that the three BAFF/APRIL receptors (BAFF-R, TACI and BCMA) have compensatory roles, suggesting a therapeutic benefit to target multiple receptors [55].

Bruton's tyrosine kinase (Btk) regulates signaling downstream of the B-cell receptor and Fcγ receptor, and it is also involved in TLR signaling. Treatment with Btk inhibitors alleviate lupus symptoms in MRL/*lpr* [56], NZB/W F1 [57,58], *B6.Sle1.Sle3* [76] and BXSB.Yaa mice [59] as well as in pristane-induced lupus [59]. Overall, based on these preclinical studies, US Food and Drug Administration-approved Btk inhibitor ibrutinib has great potential as a therapeutic agent in SLE.

Finally, two miRNAs have been identified as potent regulators of B-cell tolerance. Elevated miR-148a expression impaired B-cell tolerance by promoting the survival of immature B cells after engagement of the B-cell receptor by suppressing the expression of the autoimmune suppressor Gadd45α, the tumor suppressor Phosphatase and tensin homolog (PTEN) and the proapoptotic protein Bim. Increased expression of miR-148a facilitated the development of lethal autoimmune disease in MRL/*lpr* mice [60▪▪]. Reduction of miR-148a expression upregulated PTEN in the glomeruli and improved renal function in MRL/*lpr* mice. [77]. Conversely, miR155 is overexpressed in B cells from B6.*lpr* mice, and miR155 deletion decreased B-cell activation, autoantibody production, and autoimmune disorder [61].

Other targets

Abundant immune complexes can trigger the activation of the NLRP3 inflammasome in macrophages in SLE patients and in mouse models, leading to cell dysfunction and tissue damage [78]. In the NZB/W F1 model, a NLRP3 inhibitor termed 'Citral' alleviates lupus symptoms by inhibiting levels of reactive oxygen species, NAPDH and cyclooxygenase-2 [63]. In the pristane-induced model, a more severe lupus-like syndrome developed in mice carrying the $Nlrp3^{-R258W}$ gain-of-function mutation, providing evidence that NLRP3 plays a role in the development of SLE [64]. In a related pathway, serine/threonine kinase IL-1R-associated kinase 4 (IRAK4) is a regulator of innate immunity involved in TLR signaling. Treatment with an IRAK4 inhibitor ameliorate lupus symptom in NZB/W F1 and MRL/*lpr* mice [65]. Finally, it has been proposed that topoisomerase I plays a role in anti-dsDNA antibody

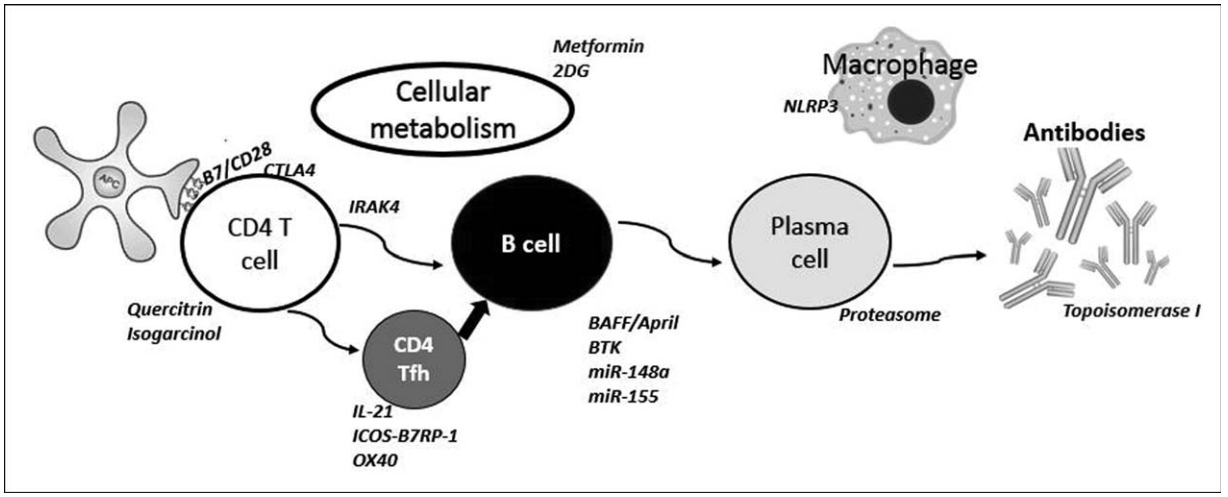

FIGURE 1. Potential therapeutic targets investigated in mouse models of systemic lupus of erythematosus.

binding, and treatment with a topoisomerase inhibitor prevented proteinuria and prolonged survival in MRL/*lpr* mice [66].

CONCLUSION

The use of murine models has led to discovery of potential therapeutic targets in diverse signaling pathways dysregulated in SLE. Immune cells, including T cells, B cells, antigen presenting cells and macrophages, are all potential targets in different models of SLE (Fig. 1). Clinical lupus is an extremely complex and diverse disease, and establishment of a mouse model with all features of the disease is very difficult. Various mouse models of SLE, spontaneous, induced or genetically engineered, have been used during the past 30 years, to answer the question of how the alteration of the immune system and target organs leads to break of tolerance to self.

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Conflicts of interest

There are no conflicts of interest.

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Metabolic abnormalities and oxidative stress in lupus

Yaima L. Lightfoot, Luz P. Blanco*, and Mariana J. Kaplan*

Purpose of review
Upon antigen exposure, immune cells rely on cell-specific metabolic pathways to mount an efficient immune response. In autoimmunity, failure in critical metabolic checkpoints may lead to immune cell hyperactivation and tissue damage. Oxidative stress in autoimmune patients can also contribute to immune dysregulation and injury to the host. Recent insights into the immune cell metabolism signatures, specifically associated with systemic lupus erythematosus (SLE) and the consequences of heightened oxidative stress in patients, are discussed herein.

Recent findings
Glucose metabolism inhibitors, mechanistic target of rapamycin pathway modulators, and peroxisome proliferator-activated receptor gamma-activating compounds demonstrate therapeutic benefit in experimental models of lupus. Mitochondrial-derived reactive oxygen species (ROS) and molecular modifications induced by oxidative stress appear to be detrimental in lupus. Effective therapies tailored toward the reconfiguration of metabolic imbalances in lupus immune cells and the reduction of mitochondrial ROS production/availability are currently being tested.

Summary
A paucity of knowledge exists regarding the metabolic needs of a number of immune cells involved in the pathogenesis of SLE, including myeloid cells and B cells. Nonetheless, SLE-specific metabolic signatures have been identified and their specific targeting, along with mitochondrial ROS inhibitors/scavengers, could show therapeutic advantage in lupus patients.

Keywords
immune cell activation, immunometabolism, oxidative stress, reactive oxygen species, systemic autoimmunity, systemic lupus erythematosus

INTRODUCTION

The emerging field of immunometabolism has provided critical insights into the metabolic changes that immune cells undergo upon activation [1,2]. Reprogramming of immune cell metabolism is required to sustain the energy demands of effector functions such as differentiation, clonal expansion, secretion of proinflammatory mediators, phagocytosis, and tissue migration. Importantly, defects in key metabolic pathways/checkpoints have been identified in autoimmunity [3,4].

Systemic lupus erythematosus (SLE) is an autoimmune syndrome characterized by dysregulated innate and adaptive immune responses and enhanced risk for multiorgan damage and cardiovascular disease [5]. As dysfunctional metabolic reprogramming can directly influence and exacerbate defective immune responses, interrogation of the metabolic status of immune cells in SLE has become a topic of interest [3]. Although recent studies have aimed to characterize the bioenergetics of differentiated/activated $CD4^+$ T cells in SLE, less is known regarding the metabolic configuration of other immune cells implicated in the pathogenesis of lupus. Moreover, SLE is associated with enhanced oxidative stress, as well as an increased prevalence of metabolic syndrome, features that also contribute to accelerated atherosclerosis and cardiovascular

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KEY POINTS

- Disease-specific immunometabolic imbalances and excess oxidative stress are characteristics of SLE.
- Reconfiguration of metabolic defects in lupus immune cell subsets has therapeutic potential in experimental models and in isolated human cells.
- A better understanding of immune cell-specific metabolic dysfunction in lupus is needed for improved treatment options.
- Excessive mitochondrial ROS production in lupus is associated with multiple pathogenic pathways in disease progression.
- Combination therapies targeting immunometabolic and mitochondrial ROS could have synergistic effects in SLE.

events in this disease. Nutrients, in addition to providing sources of energy, can also promote the activation of immune cells depending on their quality (i.e., oxidation) and availability (i.e., overnutrition). Consequently, a better understanding of the breakdown in host metabolic control, aberrant immune cell metabolism, oxidative stress, and the crosstalk between these phenomena, will be a worthwhile effort toward the development of improved treatment options for SLE patients.

In this review, we consider the most recent investigations related to immunometabolism and redox status in SLE, as well as discuss potential metabolic targets that may lead to the resolution of inflammation and the attainment of energy homeostasis in lupus patients. Where information is lacking, we explore immunometabolism-related advances in the oncology field, which may inform future areas of study in autoimmunity.

METABOLISM AND ITS REGULATION IN LYMPHOCYTES

Glucose catabolism is the primary source of ATP generation in the immune system. Although resting lymphocytes rely on oxidative phosphorylation (OXPHOS) to produce ATP, activated T and B cells shift their metabolic configuration toward aerobic glycolysis, whereby pyruvate is reduced to lactate even in the presence of oxygen. Intermediates of glycolysis can then enter the pentose phosphate pathway, where anabolic building blocks and reducing equivalents are generated. This metabolic switch is known as the Warburg *et al.* [6] effect.

The transcription factor c-Myc is critical in driving the activation-induced reprogramming of T cells by targeting a range of glycolytic genes [7]. The transcription factors estrogen-related receptor α and hypoxia inducible factor-1α, which responds to oxygen levels, also promote the expression of genes involved in metabolic reconfiguration [8–10]. Among these genes, the expression of *GLUT1*, the major glucose transporter found on T cells, is highly upregulated following T-cell receptor (TCR) engagement and under hypoxic conditions [11,12▪▪]. Indeed, T-cell-specific overexpression of Glut1 did not alter T-cell development but augmented effector functions upon activation, and ultimately resulted in signs of autoimmunity in older mice [11]. Recently, the differential expression of GLUT1 on healthy donor-derived T cells was shown to modulate their effector function, with a direct correlation noted between GLUT1 levels and TCR-induced proliferative ability, as well as with IFNγ-secretion capacity [12▪▪].

Similar to the positive transcriptional control of glycolytic genes by the aforementioned factors, negative regulators of glucose metabolism exist. Recent work by Chan *et al.* [13▪▪] demonstrates that the transcription factors PAX5 and IKZF1, which are important for B-cell development, are charged with restricting ATP levels in B cells and thus, preventing leukemic transformation. Another recently reported regulator of B-cell metabolism is the metabolic sensor Gsk3 [14▪▪], which was shown to limit glucose consumption and maintain quiescence. Along the same lines, B-cell-specific deletion of the adaptor protein Traf3 results in enhanced B-cell survival, which depends on elevated glucose metabolism and is achieved through the upregulation of Glut1 and hexokinase 2, an enzyme that catalyzes the first step in glycolysis [15▪▪]. It will be of interest to determine whether the expression of these regulatory factors is negatively affected in animal models of lupus and in patients with SLE. Indeed, B cells chronically stimulated with B-cell activating factor, a cytokine associated with SLE [16], have been shown to undergo metabolic reprogramming (i.e., enhanced glycolysis), and subsequently synthesize more antibodies [17], indicating that defects in the control of B-cell metabolism reconfiguration might influence the pathogenesis of SLE.

In addition to glucose, amino acids are important substrates for lymphocytes as they provide a source of energy as well as precursors required for *de novo* synthesis of nucleic acid and proteins [18▪▪,19▪▪,20▪]. Lipids and fatty acids (FAs) are also critical nutrients for lymphocytes; not only are they integral components of cell membranes, but they are also a high-energy source [21▪▪,22▪▪]. To date, the overarching theme in lymphocyte metabolism is that, as opposed to the glycolytic phenotype of

activated effector T cells, memory and regulatory T cells (Tregs) can also metabolize amino acids and FAs, and generate ATP through OXPHOS. In contrast, activated B cells increase glycolysis and OXPHOS equally. The details of these pathways are beyond the scope of this review and have been extensively described elsewhere [23–29].

TARGETING METABOLIC PATHWAYS IN SYSTEMIC LUPUS ERYTHEMATOSUS LYMPHOCYTES

In contrast to the glycolytic switch of activated healthy lymphocytes, repetitive TCR-engagement in lupus was previously suggested to result in the preferential use of OXPHOS through enhanced mitochondrial metabolism [30]. However, more recent work showed that SLE $CD4^+$ T cells exhibit, not only higher basal and activated mitochondrial oxidative metabolism, but also higher glycolytic activity [31▪▪]. Accordingly, combination therapy with metformin, which transiently inhibits mitochondrial respiratory-chain complex 1 resulting in downmodulation of mitochondrial respiration, and 2-deoxy-D-glucose (2-DG), a glycolytic inhibitor, ameliorated disease disorder in an experimental model of SLE [31▪▪]. Interestingly, when the reduction of pyruvate to lactate was blocked in the same mice with dichloroacetate, a drug that enhances the import of pyruvate into the mitochondria for oxidation, no improvements were noted [31▪▪]. In addition, the survival of long-lived plasma cells, which are associated with pathogenicity in SLE, was recently found to be dependent on pyruvate flux into the mitochondria [32▪▪]. These studies imply that targeting glucose oxidation (i.e., through the inhibition of the mitochondrial pyruvate complex), rather than the reversal of the Warburg effect, could be a putative effective target in SLE patients. Whether other immune cells in SLE patients metabolize glucose with the same predisposition toward oxidation remains to be tested.

In addition to dampening mitochondrial respiration, metformin also alters immunometabolism and inflammation through the activation of 5′ adenosine monophosphate-activated protein kinase (AMPK), a nutrient-sensor that modulates glucose and lipid metabolism, and brings about the negative regulation of the mechanistic target of rapamycin (mTOR). mTOR is a serine/threonine protein kinase that makes up the catalytic subunit of two protein complexes, mTOR Complex 1 (mTORC1) and mTOR Complex 2 [33]. In patients with SLE, mTORC1 activity is enhanced, and therapeutic intervention with rapamycin or *N*-acetylcysteine (NAC) prevents the proinflammatory cell death of $CD4^-CD8^-$ (double-negative) T cells and the depletion of Tregs, two reported characteristics of SLE (reviewed in [34]). Recently, metformin was also tested in *sanroque* mice ($Roquin^{san/san}$), which develop lupus-like autoimmunity due to a mutation in the ubiquitin ligase member, Roquin, a negative regulator of Tfh cells and autoantibody responses [35]. Oral administration of metformin in $Roquin^{san/san}$ mice, not only normalized T-cell responses (i.e., reduced the frequencies of Tfh and Th17 cells, whereas enhancing Treg numbers), but also suppressed the development of autoantibody-producing plasma cells and germinal center formation via the AMPK–mTOR signaling pathway [36▪]. These studies indicate that elucidating key metabolic programs involved in the pathogenesis of SLE may facilitate the modulation of multiple immune cell subsets with a single target and/or therapy.

Another factor linking metabolism and autoimmunity is the peroxisome proliferator-activated receptor gamma (PPARγ). PPARγ is a transcription factor involved in FA and glucose metabolism. Previously, pioglitazone, a PPARγ agonist and drug used to treat type 2 diabetes mellitus, was shown to be protective against vascular and renal disease in murine SLE, whereas also improving the function of $CD4^+$ T cells derived from SLE patients, suggesting a protective role of PPARγ in autoimmunity [37,38]. In a recent study, Liu *et al.* [39▪] investigated the dose effect of PPARγ expression in the immune response through genetic manipulation. Aged PPARγ hypomorphic mice developed SLE-like autoimmunity, which was T-cell-dependent, but showed a Th17 bias, as opposed to the IFNγ-association noted in human SLE T cells [38,39▪]. The therapeutic potential of pioglitazone is currently being tested in patients with SLE (NIH Clinical Research Study no. 15-AR-0060). Of note, pioglitazone is a member of a class of compounds, which have been shown to specifically inhibit pyruvate flux into the mitochondria [40]. Therefore, the protective mechanism of action of pioglitazone, in addition to PPARγ-activation, might include normalization of the aforementioned defects in pyruvate oxidation in SLE T cells.

METABOLISM OF OTHER SYSTEMIC LUPUS ERYTHEMATOSUS-ASSOCIATED IMMUNE CELLS

Given the range of autoantibodies produced in SLE, it is not surprising that significant research in the field has historically focused on the characterization of B-cell responses and B cell–T cell interactions. However, an increasing body of work has led to the

evolution of this B-cell-dominant view to include other leukocytes, such as natural killer (NK) cells, dendritic cells, macrophages, and neutrophils [41]. SLE-specific metabolic signatures have not been systematically explored in these cells; nonetheless, their activation also results in metabolic reconfiguration. For instance, human NK cells are classified by the expression of CD56, with $CD56^{dim}$ cells being the more mature and predominant circulating NK subset. It was recently reported that $CD56^{bright}$ NK cells, despite displaying lower cytotoxic capacity, are more metabolically active upon stimulation, and this allows them to produce higher levels of IFNγ [42▪]. Similarly, the metabolism of mast cells is reprogrammed once activation occurs. In response to antigen-IgE immune complexes, mast cells enhance their glycolytic capacity, and, whereas mitochondrial respiration is not enhanced, it is still required for effective degranulation of the cells [43].

The activation-induced outcomes of macrophage and dendritic cell function are also dependent on the type of metabolism adopted [44–46]. Briefly, classically activated M1 macrophages derive their energy through glycolysis, whereas alternatively activated macrophages (M2) utilize OXPHOS, particularly FA oxidation (FAO). Like M1 macrophages, activated dendritic cells upregulate their glucose uptake and lactate production; however, glycolysis provides metabolic intermediates required to fuel FA synthesis, whereas ATP is generated by OXPHOS [47]. In contrast, tolerogenic dendritic cells, like M2 macrophages, rely on FAO as their energy source [45]. Of interest, recently published data indicate that FAO is not needed for M2 polarization [48▪▪]. Indeed, disruption of FAO in myeloid cells did not change the degree of M2 polarization in response to IL-4 [48▪▪]. As the requirement of FAO in M2 macrophage polarization was originally demonstrated through chemical inhibition of FAO (i.e., with etoxomir), this study emphasizes the need for careful interpretation of experiments involving chemical inhibitors, as these might have off-target effects. Recent studies also suggest that inhibition of succinate dehydrogenase switches proinflammatory macrophage phenotype into an anti-inflammatory phenotype in which oxidative metabolism and IL-10 production are enhanced, whereas glycolysis, mitochondrial membrane potential, succinate oxidation, and mitochondrial reactive oxygen species (ROS) production are reduced [49▪▪]. Furthermore, the metabolism of plasmacytoid dendritic cells (pDCs) can be drastically alterered by type I interferons (type I-IFNs; cytokines crucial in SLE pathogenesis [50,51▪▪,52▪▪]). Indeed, exposure to type I-IFNs induces an autocrine pathway of increased OXPHOS and type I-IFN production in pDCs, which is fueled by enhanced FAO and dependent on type I-IFN receptor and PPARα signaling [53▪▪]. This amplification pathway may be of particular relevance in SLE.

The metabolic requirements of neutrophils have been relatively overlooked. This is likely due to the fact that neutrophils have few, albeit functional, mitochondria and have been shown to be primarily glycolytic [54]. However, the energy fluxes that neutrophils undergo to fulfill the energy requirements of chemotaxis, phagocytosis, and the release of neutrophil extracellular traps (NETs), remain to be fully elucidated [55–57]. The pathogen-killing ability of neutrophils is dependent on the activity of NADPH oxidase (NOX), which comprised Nox2, p22-phox, p47-phox, and p67-phox. Recently, an intriguing NOX-glycolysis activation loop was identified in stimulated neutrophils [58], as Nox2 activity was required for the stimulation-induced increase in glycolysis. The latter was also dependent on the phosphorylation of 6-phosphofructo-2-kinase (PFK-2) [58]. PFK-2 catalyzes the production of fructose-2,6-biphosphate, which activates phosphofructo-1-kinase, the rate-limiting enzyme of glycolysis. In addition, asymmetric mTOR signaling and OXPHOS were recently implicated in the chemotaxis of neutrophils [59]. In regards to NET release, metformin was previously found to inhibit NET formation, or NETosis, *in vitro* [60]. Still, whether this effect was in fact due to a direct contribution of OXPHOS to NETosis, or due to a reduction in mitochondrial ROS production and, therefore, lower generation of immunogenic oxidized mitochondrial DNA (mtDNA) [51▪▪], remains to be determined.

ROLE OF REACTIVE OXYGEN SPECIES IN TISSUE DAMAGE AND IMMUNE CELL ACTIVATION IN LUPUS

ROS have been associated with a variety of disorders due to their capacity to modify cellular components and metabolites. In lupus patients, heightened oxidative stress results in increased levels of oxidized lipoproteins, which are pathogenic *in vivo* and induce further oxidative damage [61]. In fact, lupus HDL, which tends to be oxidized in patients, lacks vasculoprotective properties and instead promotes proinflammatory responses [62]. Moreover, increased cell membrane lipid peroxidation in lupus can lead to the formation of lipid-derived reactive aldehydes (LDRA), including malondialdehyde (MDA), phosphorylcholine, and 4-hydroxynonenal (4-HNE), which can then bind to and alter proteins, rendering these immunogenic [63,64]. Counterintuitively, 4-HNE has been recently shown

to inhibit neutrophil function, including glycolysis, phagocytosis, and oxidative burst [65▪]; however, NET-formation capacity and mitochondrial ROS production were not explored. A recent study reported that the presence of LDRA-specific immune complexes correlates with disease activity in lupus patients [66], supporting a proinflammatory role of these lipid modifications. In another study, IgM antibodies against MDA and phosphorylcholine were found to negatively correlate with markers for atherosclerosis risk, in association with enhanced apoptotic cell death clearance and reduced LDRA-induced oxidative stress upon IgM binding [67▪]. Cell damage associated with excessive exposure to oxidative stress and other environmental factors can be assessed by quantification of histone H2AX phosphorylation, which is indicative of DNA double-strand breaks. In accordance with enhanced oxidative stress in lupus, H2AX phosphorylation was found to be elevated in peripheral blood cell subsets and correlated with disease activity [68]. These data implicate lipoprotein-mediated pathways and oxidative stress in particular in the increased tissue damage and propensity of cardiovascular events in lupus patients.

Conversely, a deficiency in NOX2 activity, due to a missense mutation in the p47phox (NCF1) subunit of NOX, is associated with enhanced risk to develop lupus and other autoimmune diseases [69▪▪]. Further, when greater copy numbers of *NCF1* are present, corresponding with enhanced NOX2-derived ROS, protection against lupus was reported [69▪▪]. This is akin to individuals with chronic granulomatous disease, which lack NOX activity and have an increased risk for autoimmune disease development and exhibit type I-IFN signatures [70]. Thus, the source and location of ROS production appears to be important for disease pathogenesis. Indeed, in a recent study, stimulation of neutrophils with ribonucleoprotein-immune complexes, led to increased levels of mitochondrial ROS, which resulted in mitochondria hypopolarization and the release of NETs enriched in oxidized mtDNA [51▪▪]. Low-density granulocytes (LDGs), a distinct subset of proinflammatory, NETosis-prone neutrophils present in the peripheral blood of SLE patients [71], also display enhanced mitochondrial ROS synthesis [51▪▪]. NETosis in LDGs is at least in part dependent on mitochondrial ROS production, as treatment with the mitochondrial ROS scavenger MitoTempo significantly abrogates the extrusion of NET structures in these cells [51▪▪]. Neutrophils exposed to extracellular superoxide also display enhanced NET formation and this is associated with concomitant liver injury [72]. Of consequence, the study by Lood *et al.* [51▪▪], demonstrated that oxidized mtDNA has interferogenic potential through a Transmembrane protein 173 (STING)-dependent pathway. Various cell subsets and pathways have been shown to be drivers of type I-IFN production in lupus [50,51▪▪,52▪▪,73▪▪]. Despite the pathogenic role of type I-IFNs in SLE, these cytokines are crucial in the protection against viruses. During a viral infection, mitochondrial antiviral signaling (MAVS) protein forms a complex with retinoic acid gene I (RIG-I)-like helicases to promote the expression of *type I-IFN* genes. Buskiewicz *et al.* [74▪▪] recently showed that in lupus patients, enhanced mitochondrial ROS synthesis led to RIG-I-independent MAVS oligomerization and subsequent type I-IFN production independent of infection. Taken together, these results suggest that mitochondrial dysfunction and ROS production contribute to the pathogenesis of lupus. However, the mechanisms leading to enhanced mitochondrial ROS production in SLE remain to be fully elucidated. In line with studies mentioned above, multiple mitochondria-related parameters, including ROS production, mitochondria swelling, polarization status, enzymatic activity of mitochondrial complexes, and levels of mediators of the intrinsic pathway of apoptosis, were recently demonstrated to be altered in lupus patients compared with healthy volunteers [75▪]. In lupus-prone mice, liver mitochondrial dysfunction has been shown to be controlled by mTORC1 and is, therefore, responsive to rapamycin treatment [76]. Given the reduced expression of the mitophagy facilitator dynamin-related protein 1 preceding lupus onset [76], accumulation of damaged mitochondria is a likely culprit in disease progression. Furthermore, mitochondrial Complex I dysfunction and subsequent mitochondrial ROS production induced by imiquimod (a Toll like receptor 7 agonist and mediator of lupus-like disease [77]), led to NLRP3 inflammasome activation in a recent study [78▪]. As NLRP3 inflammasome activation has been associated with SLE pathogenesis [79], the aforementioned study provides additional evidence potentially linking mitochondrial defects in lupus with downstream proinflammatory cascades that promote disease progression.

IMMUNOMETABOLISM THERAPEUTIC TARGETS AND SYSTEMIC LUPUS ERYTHEMATOSUS

Signatures corresponding with metabolic imbalances and elevated oxidative stress have been identified in the sera of lupus patients [80]. Accordingly, targeting metabolic defects in animal models of SLE with the combination of metformin and 2-DG

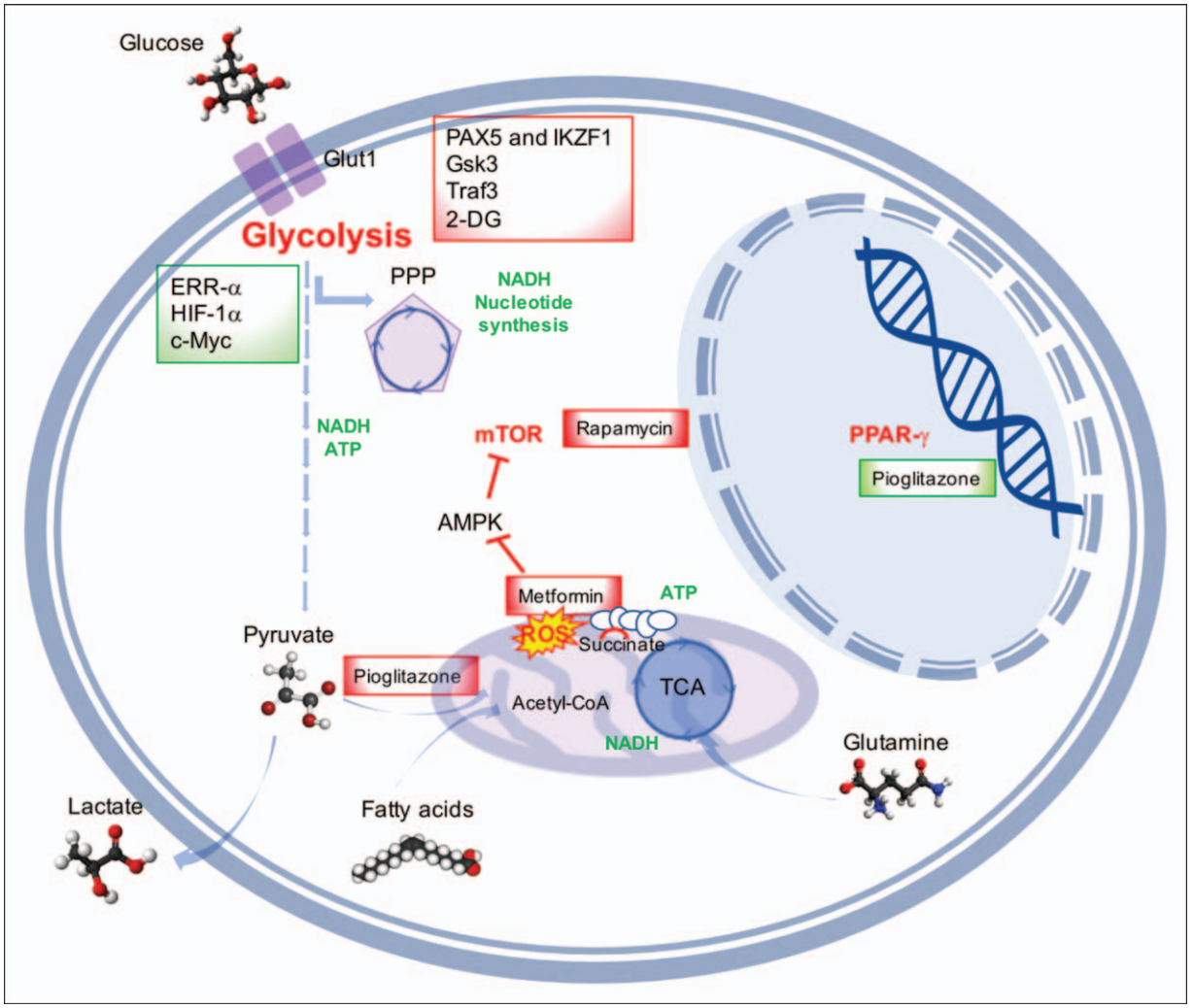

FIGURE 1. Simplified overview of the metabolism in lupus immune cells. Enhanced glucose internalization leads to subsequent incremented glycolysis, oxidation by mitochondria and penthose phosphate pathway generating reducing equivalents and nucleotides. Positive regulators are estrogen-related receptor a, hypoxia inducible factor-1a, and v-myc avian myelocytomatosis viral oncogene homolog. B-cell-specific negative regulators include: paired box 5, IKAROS family zinc finger 1, glycogen synthase kinase 3, and TNF receptor associated factor 3. 2-deoxy-D-glucose inhibits glycolysis while mitochondrial reactive oxygen species, 5′adenosine monophosphate-activated protein kinase and mechanistic target of rapamycin are inhibited by metformin. Pioglitazone activates the peroxisome proliferator-activated receptor gamma signaling pathway and prevents the transport of pyruvate into the mitochondria. Amino and fatty acids are additional fuel sources required for immune cell activation and function.

ameliorates disease disorder [31▪▪]. Similarly, mitochondrial ROS inhibition with MitoTempo reduces disease severity in a mouse model of lupus [51▪▪]. In models of skin damage due to oxidative stress, a topical mitochondria-targeted redox-cycling nitroxide mitigated the impairment [81]. Whether these therapies can improve human disease, and whether their combination has synergistic effects, remain to be determined. In a recent trial of NAC in SLE, the drug was well tolerated and reduced disease activity via the mTOR pathway [82]. Indeed, mTOR pathway modulators appear to be viable therapies, and the use of rapamycin as a therapeutic option has also been tested animal models of lupus and in humans ([34,76,83,84]; Trial ID: NCT00779194). The role of the PPARγ-agonist pioglitazone is currently being investigated in proof-of-concept trials. Information gleaned from the aforementioned studies and from ongoing investigations could identify further pathway-specific and immune cell-specific targets toward the development of personalized therapies in SLE (Fig. 1).

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Conflicts of interest

There are no conflicts of interest.

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Novel insights of microRNAs in the development of systemic lupus erythematosus

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Purpose of review
To provide a brief overview of recent progress in microRNA biogenesis and homeostasis, its function in immune system and systemic lupus erythematosus (SLE), as well as successful microRNA-based therapy *in vivo*.

Recent findings
Stepwise microRNA biogenesis is elaborately regulated at multiple levels, ranging from transcription to ultimate function. Mature microRNAs have inhibitory effects on various biological molecules, which are crucial for stabilizing and normalizing differentiation and function of immune cells. Abnormality in microRNA expression contributes to dysfunction of lupus immune cells and resident cells in local tissues. Manipulation of dysregulated microRNAs *in vivo* through microRNA delivery or targeting microRNA might be promising for SLE treatment.

Summary
Recent advances highlight that microRNAs are important in immunity, lupus autoimmunity and as potential therapy target for SLE.

Keywords
autoimmunity, microRNA, microRNA therapy, systemic lupus erythematosus

INTRODUCTION

Systemic lupus erythematosus (SLE) is an incurable autoimmune disease. During the course of disease, lupus relapse alternates with remission. Although SLE has been investigated extensively and deeply for decades, conventional drugs have only limited therapeutic efficacy and detrimental side effects like infections, infertility and hepatotoxicity frequently occur during lifetime medication [1]. Therefore, novel and specific approaches for SLE treatment should be explored and eventually employed in clinical practice.

Upon their discovery, microRNAs have drawn considerable attention due to their capability of fine-tuning gene expression with specificity and fidelity at particular development stages or immune processes [2]. Recent progress has shown that microRNA expression is tightly regulated during development, differentiation and effector phase of immune cells as well as immunological disorders. In this review, we will discuss novel findings about microRNA biogenesis and homeostasis, their role in immune system and SLE and success of microRNA-based treatment in lupus models.

REGULATION OF MICRORNA BIOGENESIS AND HOMEOSTASIS

MicroRNAs are a class of small noncoding RNAs, about 22 nucleotides in length, ubiquitously expressed in different cells and tissues [3,4]. MicroRNA biogenesis is a multistep process, which

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KEY POINTS

- Stepwise microRNA biogenesis is modulated by a complicated regulatory network, involving a large number of biological molecules.
- In different immune cells, microRNAs display significant roles in the process of cell development and function.
- The dysfunction of immune cells and resident cells in SLE is associated with several dysregulated microRNAs.
- Utilizing microRNA either as a genetic target or exogenous supplement shows curative effects on lupus mice and might provide hopeful therapeutic method for clinical use.

involves in both nucleic and cytoplasmic fine-tuned mechanism [5] (Fig. 1). Pri-miRNAs are typically transcribed by RNA polymerase II either as a single transcriptional unit or together with their host genes [6]. Microprocessors, which consist of Drosha and DGCR8, are then employed to process pri-miRNAs into microRNA precursors (pre-miRNA) [7,8]. Noncanonical mirtron bypasses Drosha cleavage and generates pre-miRNA via splicing and debranching [9,10]. Exportin 5, cooperating with Ran-GTP, exports pre-miRNA into the cytoplasm [11,12], whereas PHAX-exportin 1 pathway is responsible for m [7] G-capped pre-miRNA cytoplasmic transportation [13]. Dicer further processes pre-miRNA into a double-strand miRNA/miRNA* duplex [14]. The ds-miRNA complex is then incorporated into RNA-induced silencing complex together with Ago2, in which 'Guide' miRNA remains but 'Passenger' miRNA* is degraded [15].

Owing to a broad spectrum of targeted mRNAs, microRNAs regulate numerous physiological and pathological processes. During the last few years, new mechanisms of microRNA biogenesis have been further illustrated. MicroRNA biogenesis is exquisitely regulated at multiple levels [16]. So far, canonical pri-miRNA and noncanonical mirtron pathways have been broadly investigated. In addition, Agotron that originates from short introns but escapes Dicer cleavage has also been discovered [17]. Inside the nucleus, a histone H1-like chromatin protein, HP1BP3 retains pri-miRNA transcript on chromatin and promotes cotranscriptional pri-miRNA generation by Drosha–DGCR8 [18]. By virtue of endonuclease cleavage and polyadenylation specificactor 3 and ISY1, pri-miR-17∼92 forms an intermediate 'progenitor-miRNA' and selectively produces pre-miRNAs [19]. Adenosine deaminase (ADAR1) is capable of converting adenosine to inosine and executes RNA editing or RNAi function in the form of ADAR1/ADAR1 or Dicer/ADAR1complexes, respectively. Furthermore, in collaboration with Drosha–DGCR, ADAR1 enhances cleavage efficacy of microprocessor [20,21]. RNA-binding protein Rbfox3 is reported to inhibit the recruitment of microprocessor onto pri-miRNAs, thus downregulating pri-miRNA processsing [22]. In addition, precise pre-miRNA processing also requires biological molecule modulation. Tailor, a terminal uridyltransferase, preferentially recognizes 3′-AG of mirtron pre-miRNAs and suppresses mirtron maturation [23,24]. By contrast, TUT7/TUT4/TUT2 facilitates the terminal uridylyl addition to 1 nt-3′ overhang of Group II pre-miRNA, which produces an optimal substrate for Dicer [25]. After Dicer processing, the dsRNA is loaded onto Ago protein. Eukaryotic translation initiation factor 1A that interacts with Ago promotes Ago2 cleavage activity in RNA interference as well as Dicer-independent but Ago2-dependent miR-451 biogenesis [26]. Furthermore, a RING-E3 ubiquitin ligase Roquin is reported to regulate the homeostasis and function of mature miR-146a in $CD4^+$ T cells by binding to mature miR-146a [27▪] (Fig. 1). Mature microRNAs are then transported into intracellular compartments or extracellular fluids [28]. Irrespective of their distribution, microRNAs bind to 3′ untranslated regions of target mRNAs, following base pair principle and thereby resulting in complementary mRNA degradation or translational repression [29].

MICRORNAS IN THE REGULATION OF IMMUNE SYSTEM

MicroRNA plays an important role in regulating gene expression at the posttranscriptional level. Recent literatures have shown that microRNAs are critical for the development and function of immune system, both innate and adaptive compartment [30▪▪,31]. MicroRNA dysregulation is substantially involved in severe immune disorders, like tumor progression [32], autoimmune and autoinflammatory diseases [33].

MicroRNAs in innate immunity

Innate immune system establishes the first defense line against foreign pathogens. Immune cells like dendritic cells, macrophages and neutrophils are key components of innate immunity [34] (Table 1). Plasmacytoid dendritic cells (pDC) recognize pathogen-derived nucleic acids via toll-like receptor (TLR)7 or TLR9 and propagate downstream innate immune signaling. miR-126 that was primarily related to vascularization has been demonstrated to maintain the homeostasis and function of pDCs by

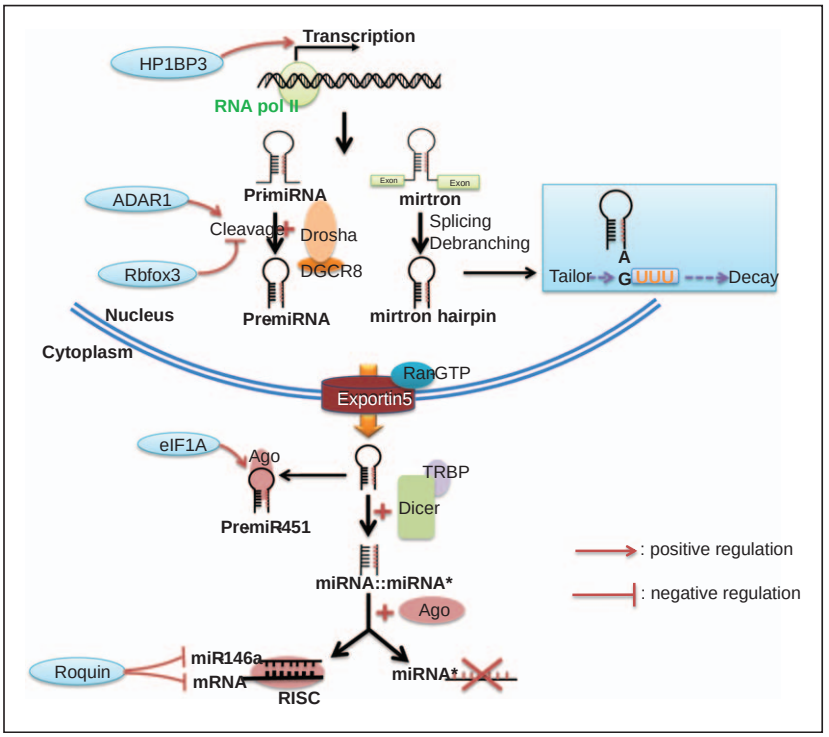

FIGURE 1. Regulation of microRNA biogenesis and homeostasis microRNAs are transcribes into hairpin-like pri-microRNAs and mirtrons by RNA pol II. After process of cleavage, Exportin5-Ran-GTP transports pre-microRNAs and mirtron hairpins from nucleus to cytoplasm. Subsequently, Dicer, together with TRBP, helps to generate the microRNA duplex, which is ultimately separated into mature microRNA and microRNA*. In the nucleus, HP1BP3 promotes the pri-miRNA transcript generation; ADAR1 upregulates and Rbfox3 downregulates pri-miRNA processing, respectively; mirtron maturation is repressed by Tailor. In the cytoplasm, EIF1A enhances Ago2 cleavage activity for pre-miR-451; the E3 ubiquitin ligase Roquin regulates mature miR-146a homeostasis by binding to microRNA itself and its targeted mRNA. ADAR1, adenosine deaminase; EIF1A, eukaryotic translation initiation factor 1A.

positively regulating mechanistic target of rapamycin activity and vascular endothelial growth factor receptor expression [35]. Antigen-presenting cells activate antigen-specific adaptive immune cells, in which microRNA plays an essential role. Th17 cells activated by lung DCs are indispensable for the pathogenesis of chronic inflammatory disease, pulmonary emphysema. miR-22 has a proinflammatory role and is robustly upregulated in $CD1a^{+}$ lung myeloid dendritic cells from pulmonary emphysema patients, while in-vitro study proves that histone deacetylase (HDAC4) is a major functional target. Strikingly, administration of locked nucleic acid (LNA) to silence miR-22 could attenuate activated lung antigen presenting cells and reserve pulmonary emphysema progression [36]. By directly targeting key signaling molecules, microRNAs in innate immune cells not only preserve innate immune homeostasis but also repress inflammatory responses.

MicroRNAs in adaptive immunity

The underlying mechanisms by which microRNAs regulate differentiation and function of adaptive immune cells, including T-cell and B-cell subsets, have also been validated as well.

T cells

$CD4^{+}$ T cells with the deletion of important components in microRNA biogenesis, including Dgcr8, Ago2 or Dicer, display effector T-cell dysregulation, which suggests that microRNAs may have critical functions in T-cell biology. Tfh cell is characterized by highly expressed master transcription factor Bcl6 and chemokine receptor C-X-C motif chemokine receptor 5. miR-155-Peli1-c-Rel pathway has been shown to regulate the late differentiation stage and function of Tfh cells. Upregulated miR-155 acts by repressing E3 ubiquitin ligase Peli1 and releases c-Rel from Peli1-mediated ubiquitination and degradation, which promotes Tfh cell proliferation and GC responses [37]. Opposite to miR-155, miR-146a inhibits Tfh cell differentiation and function by perturbing ICOS–ICOSL signaling [38]. In addition, individual members of miR-23∼24∼27 cluster has dissimilar effects on T cells. Unlike miR-24, miR-23 and miR-27 compromise Th17 and iTreg

Table 1. miRNAs in immune responses

miRNA	Cell type	Target gene (s)	Gene function	Reference (s)
miR-126	pDCs	*Tsc1*	Negative regulator of mTOR	[35]
miR-22	mDCs	*Hdac4*	Epigenetic repression	[36]
miR-155	Tfh cells	*Peli1*	E3 ubiquitin protein ligase	[37]
miR-146a	Tfh cells	*Icos*	Maintaining T-cell responses	[38]
miR-23-24-27 cluster	Th2 cells	*Il-4* *Gata3*	Inducing Th2 cell differentiation; Transcriptional activator	[39] [39,40]
miR-155	Th17 cells	*Jarid2*	Transcriptional repressor	[41]
miR-183-96-182 cluster	Th17 cells	*Foxo1*	Transcriptional regulator	[42▪]
miR-17-92 cluster	B cells	*Pten*	Tumor suppressor	[43]
miR-148a	B cells	*Pten* *Bim* *Gadd45α*	Tumor suppressor Apoptotic activator Autoimmunity suppressor	[44]

mTOR, mechanistic target of rapamycin.

differentiation. Moreover, Th2 differentiation is inhibited by miR-24 and miR-27 by targeting *IL-4* and *GATA3*, respectively [39]. These two microRNAs collaboratively hamper IL-4 production in Th2-Type immune response and in-silico analysis further reveals a series of target genes [40]. Th17 differentiation is prominently associated with miR-155-Jarid2 pathway. Th17 cells devoid of miR-155 have elevated Jarid2 protein that recruits Polycomb Repressive Complex 2 and silences Th17-related genes [41]. In addition, pathogenic Th17 cell differentiation and effector function are controlled by miR-183-96-182 cluster. IL-6-signal transducer and activator of transcription (STAT)3 signaling elicits miR-183 cluster which directly targets the transcription factor *Foxo1*. Downregulated Foxo1 expression augments the synthesis of proinflammatory cytokine receptor IL-1R1 and ultimately promotes the pathogenicity of Th17 cells [42▪].

B cells

B-cell development requires a sequential process, from pre-B cells, pro-B cells and immature B cells to finally mature B cells. Similar to T-cell subsets, emerging evidence reveals that microRNAs play essential roles in B-cell development. 'IgM^b-macroself' mouse model harbors no mature B cells in the spleen and lymph nodes and is extensively utilized in B-cell development studies [43]. miR-19, a member of miR-17∼92 cluster, functions as a negative regulator of B-cell central tolerance by repressing the expression of *Pten*. Besides, miR-17 accounts for the pro-B to pre-B transition in early B-cell development through other molecular pathways, whereas plausible targets like *Pten, Phlpp2 and Bim* are excluded [43]. Using the same 'IgM^b-macroself' model, another research demonstrates that miR-148a regulates B-cell tolerance as well. The cognate binding sites on *Gadd45α, Pten and Bim* transcripts are targeted by miR-148a. Further study shows that upregulated miR-148a in MRL-lpr mice acts as a possible cause of lupus progression [44]. Accordingly, great advances might be made in discovering potent therapeutic targets against intractable autoimmune diseases, with the exploration of underlying mechanisms about B-cell tolerance. And it is evident that individual microRNAs in one cluster have significant impacts on adaptive immunity in synergistic or antagonistic ways, implying that defective microRNA regulation might have pivotal roles in immune-related diseases.

MICRORNAS IN SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus is a multifactorial autoimmune disease, characterized by pathogenic autoantibodies production and multiorgan/system involvement [45]. Of note, renal disease possesses marked morbidity and mortality [46]. It is well established that the immunopathology of lupus nephritis (LN) features immune-complex deposition and cell proliferation [47]. Given the versatile functions of microRNAs in immune responses, studies on microRNAs might further illustrate the pathogenesis of SLE, provide novel biomarkers and potential therapeutic strategies.

MicroRNA-mediated immune cell dysfunction

Immunopathogenesis of SLE is mainly associated with innate and adaptive immune system dysfunction [48], in which pDCs, B cells and T cells are involved.

Table 2. miRNAs expression in SLE

Location	miRNA	Level	Functional target (s)	Reference (s)
Immune cells				
Dendritic cell	miR-155	↑	*Ship1*	[53]
	Let-7c	↑	*Blimp1, Socs1*	[54]
B cell	miR-155	↑	*Ship1, Acida*	[55,57]
	miR-30a	↑	*Lyn*	[56]
	miR-181b, miR-361	↑	*Acida*	[57]
	miR-23b, miR-30a, miR-125b	↑	*Blimp1*	[57]
T cell	miR-29b	↑	*Sp1*	[58]
	miR-31	↓	*RhoA*	[59]
	miR-142-3p/5p	↓	*IL-10, CD84, SAP*	[60]
	miR-125a	↓	*Stat3, Ifng, Il13*	[61]
	miR-98	↑ (by GCs)	*IL-3, Fas, FasL, TNFRSF1B*	[62]
Resident cells				
Renal tubular cell	miR-130b	↑	*Erbb2ip*	[63]
Mesangial cell	miR-148a-3p	↑	*Pten*	[64]
	miR-150	↑	*Socs1*	[65]
	miR-744	↑	*Pip1b*	[66]
	miR-26a, miR-30b	↓ (by HER2)	———	[67]
	miR-422a	↑	*KLK4*	[68]
Podocyte	miR-26a	↓	Actin, vimentin	[69]

SOCS, suppressor of cytokine signaling.

Activated pDCs constitutively produce type I interferon, which displays a central role in lupus onset and progression [49]. Consistently, type I interferon signature in SLE patients are positively correlated with disease severity [50]. Previous studies have clarified that miR-146a in SLE patients downregulates type I interferon pathway through targeting interleukin1 receptor associated kinase 1/TNF receptor associated factor 6, interferon regulatory factor (IRF)-5 and STAT-1 [51]. Of note, type I interferon reciprocally inhibits miR-146a generation by accelerating the production of DICER inhibitor-MCPIP1 (zinc finger CCCH type containing 12A) which hampers miR-146a maturation [52] (Table 2). Being an antigen-presenting cell, distinct aspects of pDCs are modulated by microRNAs. TLR7 stimulation activates miR-155-*Ship*1 pathway and increases CD40 on pDCs derived from lupus mice [53]. Blimp1–let-7c circuit, in which let-7c inhibits Blimp1 and Suppressor of Cytokine Signaling (Socs)1 expression, regulates proinflammatory cytokines secretion from pDCs [54]. miR-155 exhibits diverse effects on B-cell development in germinal centers. miR-155$^{-/-}$*Fas*^{lpr} mice display mitigated lupus phenotype, including lower IgG autoantibodies and alleviative renal lesion. Absent miR-155 rescues the negative regulator SHIP-1, thus repressing proliferation and function of B cell [55]. In addition, elevated miR-30a in lupus peripheral B cells posttranscriptionally downregulates Lyn. Depressed Lyn contributes to lupus B-cell hyperactivity [56]. Of note, HDAC inhibitor can alleviate disease severity and autoantibody responses in MRL-lpr mice in that several microRNAs that are elicited can silence AID and Blimp-1 and disrupt B-cell maturation [57].

Increased miR-29b [58], miR-21, miR-148a and miR-126 in lupus CD4$^+$ T cells repress Dnmt1 and thus make great contributions to T-cell autoreactivity by fine-tuning DNA methylation. Lower IL-2 production in lupus is partially ascribed to down-regulated miR-31 in lupus T cells. RhoA, restored by attenuated miR-31 alters nuclear factor of activated T-cells expression and weakens IL-2 promoter activity [59]. Moreover, downregulated miR-142-3p/5p results in derepression of IL-10, CD84 and SAP (SH2 domain containing 1A), which further leads to lupus T-cell hyperactivity and B-cell hyperstimulation [60]. The disruption of Treg-mediated homeostasis is causal for autoimmunity. MiR-125a maintains the immunosuppressive capacity of Treg cells and is down-regulated in lupus CD4$^+$ T cells. Genes encoding effector T-cell factors like *Stat3, Ifng* and *Il13* are significantly suppressed by miR-125a [61]. Intriguingly, conventional medicine like glucocorticoids can influence the expression of several microRNAs, partially accounting for their pharmacological actions in SLE treatment [62].

MicroRNA-mediated resident cell dysfunction

Pathogenic autoantibodies in SLE combine with self-antigens to form immune complexes, which

Table 3. miRNA-based therapy *in vivo*

miRNA	Model	Administration	Therapeutic effects	Reference
miR-130b	(NZB × NZW) F1 mice + IFNα	miR-130b agomir	↓proteinuria, ↓immune complex deposition	[70▪▪]
miR-146a	BXSB mice	MS2-miR-146a VLPs	↓autoantibodies, ↓proinflammatory cytokines	[71]
miR-21	B6.Sle123 mice	miR-21 LNA	↓splenomegaly, ↓CD4/CD8 T-cell ratio	[72]
miR-155	B6 mice + Pristane	miR-155 antagomir	↓DAH, ↓proinflammatory cytokines	[73▪▪]

DAH, diffuse alveolar hemorrhage; HDAC4, histone deacetylase; LNA, locked nucleic acid; pDC, plasmacytoid dendritic cells; SLE, systemic lupus erythematosus.

could subsequently deposit in local tissues or circulate in body fluids. Other than immune cells infiltration, glomerular cells are also responsible for LN development. MiR-130b-3p promotes epithelial-mesenchymal transition by targeting ERBB2IP which negatively regulates transforming growth factor (TGF-β)-mediated epithelial to mesenchymal transition [63]. Highly expressed miR-148a-3p in LN glomeruli promotes mesangial cell proliferation by directly inhibiting PTEN [64]. In renal proximal tubule cells and mesangial cells, upregulated miR-150 results from TGF-β1 stimulation. miR-150 afterward targets SOCS1, facilitating the synthesis of profibrotic proteins and accelerating renal fibrosis [65]. Type I interferon is highlighted in renal inflammatory responses and resident cells injury. Targeting phosphatase protein tyrosine phosphatase, non-receptor type 1, miR-744 in renal mesangial cells exaggerates type I interferon signaling pathway [66]. Down-regulation of miR-130b in LN kidney is inversely correlated with type I interferon signature, whereas *IRF-1* is identified as its direct target. Upon IFNα and IRF1 induction, erb-b2 receptor tyrosine kinase 2 (HER2) preferentially overexpresses in LN. Mesangial cell proliferation inhibitor, miR-26a and miR-30b, is downregulated by HER2. It is of central interest that anti-HER2 therapy is plausible in LN [67]. In formalin-fixed, paraffin-embedded kidney specimens from LN patients, miR-422a is significantly elevated. Kallikrein-related peptidase 4 is lessened because of miR-422a's direct repression, suggesting the involvement of local factors in LN disorder [68]. Furthermore, miR-26a decrease in podocyte causes cell injury via repressing actin and vimentin [69]. Taken together, those studies emphasize the significant roles of microRNAs in resident cells from targeted tissues.

MicroRNA-based therapy for systemic lupus erythematosus

MicroRNA-based therapy attracts intensive interest as individual microRNAs in different cells may target distinct functional genes. Indeed, in-vivo studies of lupus mice demonstrate that therapeutic strategies, involving exogenous microRNA addition or pathogenic microRNA elimination, are efficacious and convenient (Table 3). On one hand, artificial microRNA replenishment via delivery of exogenous microRNA or agomir exerts curative effects. Reduced miR-130b negatively regulates type I interferon signaling pathway in lupus resident mesangial cells. After miR-130b agomir injection, LN mice have less proteinuria, immune-complex deposition and renal pathological changes [70▪▪]. Intravenous injection of MS2-miR-146a VLPs into BXSB mice increases mature miR-146a in periferal blood mononuclear cells, kidney, lung and spleen. Subsequently, significantly decreased pathogenic autoantibodies and proinflammatory cytokines are observed [71]. On the other hand, diminishing pathogenic microRNAs is also a useful tactic. Short-term LNA intravenous injection to silence miR-21 expression successfully de-represses its target programmed cell death 4, whereas long-term intraperitoneal injection ameliorates splenomegaly, one of the cardinal autoimmunity manifestations in B6.Sle123 mice [72]. In addition, in accordance with miR-155$^{-/-}$ mice, diffuse alveolar hemorrhage progression is attenuated by miR-155 antagomir. The administration of miR-155 antagomir is executed before/after pristane injection. Therefore, effectively antagonizing miR-155 *in vivo* is potentially a preventive and therapeutic method for lupus pulmonary inflammation [73▪▪]. Notwithstanding those successful animal trials of microRNAs-centered therapy for SLE, numerous attempts are still needed for clinical development. It is inspiring that at present, microRNA therapeutics for cancer, diabetes, scleroderma and hepatitis C are in clinical trials [74]. With more specific mechanisms about microRNA biogenesis and regulation being expounded, searching for more potent and powerful microRNA candidates is up-and-coming. Meanwhile, several outstanding issues such as pharmacokinetics/pharmacodynamics profiles, possible drug toxicities as well as efficient delivery systems [75] remain to be elucidated.

CONCLUSION

There is no doubt that microRNAs are crucial for SLE development. Individual microRNAs in different

cell subsets target various molecular pathways, which in turn demonstrate the importance of miRNAs in a broad range of immune and autoimmune processes. Past few years have witnessed great progress in clarifying microRNA's role in the pathogenesis, diagnosis and treatment of SLE, whereas further attempts are extremely urgent to push forward microRNA-related biomarkers and therapy into clinical use.

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Conflicts of interest

There are no conflicts of interest.

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Antiphospholipid syndrome: an update for clinicians and scientists

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Purpose of review
Antiphospholipid syndrome (APS) is a leading acquired cause of thrombosis and pregnancy loss. Upon diagnosis (which is unlikely to be made until at least one morbid event has occurred), anticoagulant medications are typically prescribed in an attempt to prevent future events. This approach is not uniformly effective and does not prevent associated autoimmune and inflammatory complications. The goal of this review is to update clinicians and scientists on mechanistic and clinically relevant studies from the past 18 months, which have especially focused on inflammatory aspects of APS pathophysiology.

Recent findings
How antiphospholipid antibodies leverage receptors and signaling pathways to activate cells is being increasingly defined. Although established mediators of disease pathogenesis (like endothelial cells and the complement system) continue to receive intensive study, emerging concepts (such as the role of neutrophils) are also receiving increasing attention. In-vivo animal studies and small clinical trials are demonstrating how repurposed medications (hydroxychloroquine, statins, and rivaroxaban) may have clinical benefit in APS, with these concepts importantly supported by mechanistic data.

Summary
As anticoagulant medications are not uniformly effective and do not comprehensively target the underlying pathophysiology of APS, there is a continued need to reveal the inflammatory aspects of APS, which may be modulated by novel and repurposed therapies.

Keywords
antiphospholipid syndrome, complement, endothelial cells, neutrophils, pregnancy loss, thrombosis

INTRODUCTION

Vascular complications, including thrombotic events, are among the leading causes of morbidity and mortality in lupus. Antiphospholipid antibodies (aPL), a major driver of thrombosis risk, are present in up to one-third of lupus patients. When aPL are associated with certain clinical complications (either thrombotic or obstetric), a diagnosis of antiphospholipid syndrome (APS) is assigned (Table 1) [1]. Beyond lupus-associated APS, approximately half of APS cases will be diagnosed as a standalone syndrome (i.e., primary APS) [2].

APS is a leading acquired cause of thrombosis and pregnancy loss, with an estimated prevalence of one in 2000 [3]. Framing this risk another way, aPL can be detected on the order of 10% of the time in the setting of certain events, including pregnancy morbidity, stroke, myocardial infarction, and deep venous thrombosis [4]. Emphasizing the systemic nature of APS, the diagnosis also portends risk for cytopenias (especially hemolytic anemia and thrombocytopenia), mitral and aortic valve lesions, seizure disorder, accelerated cognitive decline, and nephropathy in the form of thrombotic microangiopathy [5]. The approach to treatment is typically with anticoagulant drugs, which are not uniformly effective in preventing recurrent aPL-mediated thrombosis and pregnancy loss and offer insufficient protection against the varied 'noncriteria' manifestations of APS. Indeed, 44% of 'triple-positive' APS patients (positive testing for anticardiolipin, antibeta-2-glycoprotein I, *and* lupus anticoagulant) will develop recurrent thrombosis

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KEY POINTS

- Current standard-of-care therapy for APS does not explicitly target inflammatory aspects of APS pathophysiology.
- A better understanding of intercellular and intracellular signaling pathways in APS has revealed potential drug targets (i.e., interferons, phosphoinositide 3-kinase, etc.).
- In addition to the well established cellular mediators of APS pathogenesis (endothelial cells, platelets, etc.), there is emerging interest in the contribution of myeloid-lineage cells. The role of neutrophil extracellular trap release, in particular, warrants further study.
- Complement activation and deposition continue to be recognized for their role in APS pathogenesis. Activity of this pathway may be mitigated by several medications, including rivaroxaban and hydroxychloroquine.
- Adjuvant therapeutics, including statins and hydroxychloroquine, have the potential to improve APS pregnancy outcomes, based upon animal studies and small clinical trials.

over a 10-year follow-up period (even with the majority being prescribed anticoagulants) [6]. Furthermore, at least 20% of obstetric APS patients have adverse outcomes in spite of therapy with aspirin and low-molecular-weight heparin [7].

Despite its high prevalence and potential for devastating morbidity, APS pathophysiology has yet to be fully defined. APS was historically viewed as a coagulation problem; however, clinical observations and basic science discoveries are increasingly highlighting a more multifaceted syndrome with an associated (and perhaps even central) inflammatory component [8]. Herein, we will discuss recent discoveries over the past 18 months, which have continued to increase our understanding of APS pathophysiology. We will also discuss how this improved basic understanding may translate to new and repurposed therapeutics for APS (Table 2).

CELL ACTIVATION AND SIGNALING PATHWAYS: NEW CONCEPTS

Understanding the cellular signaling pathways that mediate APS pathogenesis has remained somewhat elusive, at least partially the consequence of study heterogeneity. Studies have utilized different types of aPL (monoclonal vs. patient-derived; protein cofactor-dependent vs. cofactor-independent) and have focused on a variety of cellular targets (endothelial cells, platelets, monocytes, neutrophils, trophoblast cells, etc.).

Many pathogenic antibodies in APS do not target phospholipids themselves, but rather phospholipid-binding protein cofactors. The best characterized of these cofactors is beta-2 glycoprotein I (β_2GPI), a lipid-binding protein present at high levels in plasma [22,23], albeit with largely unknown endogenous function. The mechanistic schema is that anti-β_2GPI antibodies potentiate thrombosis by engaging β_2GPI protein that has been recruited to cell surfaces – and thereby promote cell activation [24–26]. The mechanisms by which anti-β_2GPI antibodies activate cells have been recently reviewed [27], with roles especially suggested for the cell surface proteins annexin A2, apolipoprotein E receptor 2 (ApoER2), Toll-like receptor 2 (TLR2), and TLR4 [27].

Table 1. Classification criteria for antiphospholipid syndrome

APS is present if one of the clinical criteria and one of the laboratory criteria are met		
Clinical criteria	1. Vascular thrombosis	≥1 clinical episode of arterial, venous, or small-vessel thrombosis
	2. Pregnancy morbidity	(a) ≥1 unexplained death of a morphologically normal fetus at ≥10 weeks of gestation
		(b) ≥1 premature delivery of a morphologically normal fetus at <34 weeks gestation because of:
		(i) Severe preeclampsia or eclampsia defined according to standard definition
		(ii) Recognized features of placental insufficiency
		(c) ≥3 unexplained consecutive miscarriages at <10 weeks gestation, with maternal and paternal factors (anatomic, hormonal, or chromosomal abnormalities) excluded
Laboratory criteria	The presence of antiphospholipid antibodies on ≥2 occasions ≥12-week apart:	
	(a) Presence of lupus anticoagulant in plasma	
	(b) Medium-titer to high-titer anticardiolipin antibodies of IgG or IgM isoforms	
	(c) Medium-titer to high-titer antibeta-2 glycoprotein-I antibodies of IgG or IgM isoforms	

Adopted from [1].

Table 2. Summary of efficacy and mechanisms by which repurposed therapeutics could potentially benefit antiphospholipid syndrome patients

		Hydroxychloroquine	Statins	Rivaroxaban
Summary of efficacy				
Thrombotic risk	Mouse models	Protects [9,10▪▪]	Protects [11]	
	APS patients	No prospective studies in APS, but protects in postoperative setting [12]	No studies in APS, but protects in the general population [13]	Efficacy may be similar to warfarin in carefully-selected patients (though further study is needed) [14▪▪]
Obstetric events	Mouse models	Prevents fetal death and metabolic changes [15▪]	Prevents fetal death [16]	
	APS patients	May prevent pregnancy loss [7,17]	May prevent fetal morbidity and mortality [18▪▪]	
Potential anti-inflammatory mechanisms				
Complement		Inhibits activation and deposition [15▪]		Decreases activation [19▪]
Type I IFN signature		Decreases [20▪▪]	Decreases [20▪▪]	
NET release		Possibly inhibits [21]		

ASP, antiphospholipid syndrome; IFN, interferon; NET, neutrophil extracellular trap.

ApoER2 (also known as LDL receptor-related protein 8) is one receptor for β_2GPI (and consequently β_2GPI-dependent aPL) on monocytes, endothelial cells, and platelets. Indeed, in a 2011 study, Ramesh *et al.* [28] demonstrated ApoER2$^{-/-}$ mice are relatively resistant to thrombosis when confronted with aPL. More recently, it has been revealed that ApoER2 may play an important role in obstetric APS [29]. Specifically, Ulrich *et al.* [29] demonstrated enhanced placental trophoblast cell proliferation and migration *in vitro* when aPL engage β_2GPI/ApoER2 complexes on the trophoblast cell surface. Extending these studies to an in-vivo model of aPL-mediated pregnancy loss, they demonstrated protection in ApoER2$^{-/-}$ mice [29]. In another recent study, Mineo *et al.* [30▪] developed a mAb against β_2GPI that prevents pathogenic aPL binding, thereby protecting against aPL-mediated cell activation. The antibody attenuated the association of β_2GPI with ApoER2, thereby normalizing endothelial and trophoblast cell function *in vitro*, as well as preventing thrombosis and fetal loss *in vivo* [30▪]. Although further study is clearly needed, the intersection of aPL, β_2GPI, and ApoER2 warrants investigation as a potential therapeutic target in patients.

As neither β_2GPI itself, nor some β_2GPI 'receptors' such as annexin A2, have a cytoplasmic domain to mediate signaling, there has been interest in additional partner proteins that may convey activating signals to the cytoplasm. On this front, particular attention has been given to the cell-surface TLRs, TLR2 and TLR4. In mouse models, TLR4 deletion protects against venous and arterial thrombosis in some [31–33], but not all [34▪], studies (it is worth pointing out that the latter study utilized cofactor-independent aPL). Studies of obstetric APS have also yielded mixed results with an older study demonstrating no role for TLR4 in an in-vivo model of pregnancy loss [35]. In contrast, Azuma *et al.* [36] recently suggested that, at least in-vitro, TLR2 and TLR4 facilitate inflammatory cytokine production by trophoblast cells in response to anti-β_2GPI antibodies.

Signaling pathways downstream of the aforementioned receptors, at least as they relate to APS pathogenesis, remain incompletely understood. Terrisse *et al.* [37▪] recently investigated downstream signaling pathways by which aPL (especially IgG isolated from APS patients) activate platelets. The authors demonstrated that aPL potentiate ex-vivo platelet activation through surface glycoprotein Ibα (the platelet receptor for von Willebrand factor) and TLR2, by a mechanism involving class IA phosphoinositide 3-kinase (PI3K) α and β isoforms [37▪]. One downstream consequence of PI3K signaling is activation of the serine/threonine kinase Akt, a pathway that supports cell survival, proliferation, and migration [37▪]. Indeed, PI3K inhibitors, which are being explored as potential drug targets in other contexts [38], are effective at preventing aPL-mediated platelet activation [37▪]. Interestingly, another study has suggested that Akt activation is a downstream consequence of trophoblast cell activation by aPL [29].

Beyond the engagement of aPL with cell surfaces, a recent report by Wu *et al.* [39▪] suggests an intriguing new mechanism by which aPL-activated endothelial cells may propagate this activation in

paracrine fashion to other endothelial cells. Anti-β_2GPI antibodies trigger the release of 'extracellular vesicles' from endothelial cells, which the authors define as inclusive of both microparticles and exosomes [39▪]. These vesicles then activate endothelial cells through a mechanism that is not dependent upon packaged cytokines such as IL-1, but rather single-stranded RNA that signals through TLR7 in the recipient cell [39▪]. They also speculate that these vesicles may be a mechanism for delivery of specific and functionally-relevant micro-RNA, though this hypothesis requires further study.

THE VESSEL WALL: ENDOTHELIAL PROGENITORS AND INTERFERONS

Our group recently looked 'upstream' of endothelial cells, asking whether a deficiency in reparative, circulating endothelial progenitors might contribute to defective maintenance and health of the endothelium over time. Indeed, a deficiency in the number and function of such progenitors is a well recognized aspect of both lupus and rheumatoid arthritis (RA) [40]. We found that primary APS patients have a reduction in functional endothelial progenitors, which was interestingly not dependent upon patient IgG; rather, we discovered a type I interferon signature in the APS patients, abrogation of which could restore normal progenitor function [41▪]. These findings were replicated by van den Hoogen *et al.* [20▪▪], who found that approximately 50% of primary APS patients have a type I interferon signature, which was less likely to be present in patients taking either hydroxychloroquine or statins. Interestingly, they also found that the interferon signature correlated with expansion of 'intermediate' and 'nonclassical' monocytes (which have been previously linked to cardiovascular disease in lupus and RA) [20▪▪]. How these monocytes intersect with endothelial progenitors [42], and whether there is a role for anti-interferon therapy in APS [43], are questions that deserve further consideration.

One potential consequence of endothelial cell (and progenitor) dysfunction is atherosclerosis, an accelerated version of which is a well known complication of lupus [44], and which has also been reported in APS [45,46]. The recent work of Benagiano *et al.* has examined the role of T_H1 specific inflammatory responses to β_2GPI in established atherosclerotic lesions of primary APS patients. Their work demonstrated that plaque-derived, β_2GPI-specific CD4+ T-lymphocytes facilitate perforin-mediated and Fas ligand-mediated cytotoxicity, pointing to a role for these autoreactive T cells in plaque destabilization (and potentially the arterial thrombotic events that are known to occur at higher frequency in APS) [47▪▪]. They also demonstrated that β_2GPI can induce proliferation of (and IFN-γ expression by) plaque-derived T-cell clones [47▪▪]. Furthermore, these T cells amplify monocyte responses, such as the production of tissue factor (TF) and matrix metalloproteinases, which can be inhibited with an anti-IFN-γ antibody [47▪▪].

MYELOID-LINEAGE CELLS: NEUTROPHIL EXTRACELLULAR TRAPS AND MONOCYTE NOX2

The role of neutrophils in APS pathogenesis has only recently been investigated. This interest was precipitated by emerging descriptions of neutrophils as mediators of both pathologic clotting and autoimmune diseases [48,49]. In particular, neutrophil extracellular traps (NETs) (extracellular chromatin-based structures released by activated neutrophils) have been described as triggers of autoimmunity and tissue damage, as well as important instigators of thrombosis [50].

With this background in mind [51], our group recently identified increased levels of cell-free DNA and NETs in the circulation of primary APS patients, as compared with healthy controls [52▪▪]. When APS neutrophils were cultured *in vitro*, they demonstrated an enhanced propensity to spontaneously release NETs [52▪▪]. Mechanistically, anti-β_2GPI IgG appears to be at least one factor in patient blood that supports NET release, with the mechanism dependent upon both TLR4 and formation of reactive oxygen species [52▪▪]. Furthermore, the prothrombotic potential of aPL-mediated NETs was demonstrated in a thrombin generation assay, with this potential abrogated by treatment with deoxyribonuclease (DNase) [52▪▪]. In parallel to our work, van den Hoogen *et al.* [53] reported increased levels of circulating 'low-density granulocytes' or LDGs in patients with primary APS. This proinflammatory subset of neutrophils has been well characterized in systemic lupus erythematosus and other autoimmune disorders, in which they are reported to release NETs in exaggerated fashion [54]. Whether LDGs are important sources of NETs in APS awaits further study [55].

The in-vivo relevance of NETs was recently confirmed by our group in a mouse model of APS. In this model, IgG from triple-positive APS patients potentiated venous thrombosis in mice that had been subjected to flow restriction in the inferior vena cava by a standard surgical stenosis [56▪]. As compared with control mice, mice treated with APS IgG were twice as likely to develop macroscopic thrombi

in response to flow restriction. Mechanistically, APS thrombi were enriched for NETs, whereas patient IgG could be detected on the surface of circulating neutrophils [56▪]. Furthermore, APS IgG-mediated thrombosis could be reversed by either neutrophil depletion or administration of systemic DNase [56▪]. Around the same time, Manukyan *et al.* [34▪] published an elegant study demonstrating that cofactor-independent aPL could similarly potentiate thrombosis in an inferior vena cava flow-restriction model. Their interesting work found a major role for leukocyte activation in thrombus formation, which could be abrogated by deletion of NOX2 (the catalytic subunit of NADPH oxidase) from bone marrow-derived cells. Although the authors' primary interest was in monocyte NOX2 and its role in TF expression, there is also a well accepted role for neutrophil NOX2 in NET formation [57]. Further studies may assess the role of these cofactor-independent antiphospholipid antibodies in inducing NET release *in vitro* and *in vivo*.

COMPLEMENT: AT THE INTERSECTION OF COAGULATION AND INFLAMMATION IN ANTIPHOSPHOLIPID SYNDROME

Animal models of APS have supported a role for complement activation in both thrombotic events and pregnancy loss [58,59]. Studies in APS patients have demonstrated smoldering activity of the complement cascade [60–62], whereas a recent case report revealed deposition of β_2GPI protein, IgG, and complement components C1q, C4, C3, and C5b-9 at the endothelial surface of an occluded artery in an APS patient [63]. Furthermore, this patient, who had suffered recurrent arterial occlusions, was successfully revascularized while under treatment with eculizumab, a terminal complement inhibitor [63].

In lupus, antibodies to C1q (a complex that initiates the complement cascade in response to immune complexes) amplify complement activation and strongly correlate with certain clinical manifestations such as proliferative nephritis [64]. Oku *et al.* [65] recently investigated these antibodies in primary APS patients, demonstrating that 36% of patients had detectable anti-C1q (compared with 55% of lupus patients). Interestingly, titers of anti-C1q were significantly higher in patients with refractory APS [65].

Rivaroxaban, a direct factor Xa inhibitor, has recently been considered as an alternative agent to vitamin K antagonists in APS. The first randomized, prospective study investigating use of rivaroxaban in APS (RAPS trial) was recently published. In patients with a history of venous thromboembolism (who had already demonstrated stable disease on warfarin), both warfarin and rivaroxaban prevented new thrombotic events for 210 days in every study patient [14▪▪]. Bleeding events and overall adverse events were also similar between the groups [14▪▪]. Although a full recounting of this important trial is beyond the scope of this brief review, we would refer you to a detailed comment on the topic [66]. Related to our discussion of the complement pathway, a post-hoc analysis of the RAPS trial revealed that, prior to randomization, APS patients had significantly higher markers of complement activation as compared with normal controls [19▪]. Although patients in the warfarin group showed stable elevation of these markers over time, patients randomized to rivaroxaban demonstrated decreased C3a, C5a, and soluble C5b-9 (all markers of classical pathway activation) [19▪]. In contrast, the alternative pathway marker, Bb, was unchanged with rivaroxaban treatment [19▪]. Whether direct oral anticoagulants have additional anti-inflammatory properties is a topic that certainly warrants further study.

REPURPOSING MEDICATIONS: STATINS AND HYDROXYCHLOROQUINE AS ADJUVANT THERAPIES IN ANTIPHOSPHOLIPID SYNDROME?

HMG-CoA reductase inhibitors (or statins) have long been recognized to have pleotropic anti-inflammatory effects supportive of vascular health, including reductions in inflammation, oxidative stress, and coagulation [67]. Clinically, statins appear to reduce the risk of venous thromboembolism in the general population [13]. In mouse models of APS, statins mitigate aPL-mediated thrombotic events and fetal death [11,16]. Furthermore, when administered to APS patients, statins decrease both prothrombotic and proinflammatory biomarkers [68].

The standard of care for managing pregnancy complications in APS is the administration of low-dose aspirin and low-molecular-weight heparin (the latter at either prophylactic or therapeutic doses, depending on the patient's thrombosis history) [69,70]. However, as detailed in recent review articles [69,70], pregnancy complications in APS are often not based in frank placental thrombosis, but rather spiral artery vasculopathy, as well as acute and chronic inflammation – with increased infiltration of inflammatory cells and deposition of complement in the placentae of women with APS [71–73]. Lefkou *et al.* [18▪▪] recently investigated the use of pravastatin in refractory obstetric APS. In their clinical trial, 21 patients with refractory obstetric APS (emergence of preeclampsia and/or intrauterine growth restriction [IUGR] despite treatment with

low-dose aspirin and low-molecular-weight heparin) were randomized either to continue standard therapy or to receive pravastatin 20 mg/day at the onset of preeclampsia/IUGR [18▪▪]. There was a remarkable therapeutic benefit, with all the patients receiving pravastatin delivering healthy infants at 34–38 weeks [18▪▪]. In contrast, the 10 patients who remained on standard therapy had three stillbirths at 25–26 weeks and seven preterm Cesarean sections (resulting in two fetal deaths) [18▪▪].

Hydroxychloroquine (which is nowadays prescribed to essentially all patients with lupus) was utilized in the 1970s to reduce the risk of venous-thromboembolism in postoperative patients [12]. In the 1990s, hydroxychloroquine was demonstrated to protect against aPL-mediated thrombosis in mice [9]. Furthermore, there have been hints of a reduction in thrombosis risk in lupus patients taking hydroxychloroquine, as compared with those who are not [74,75]. Mechanistically, a recent study demonstrated that hydroxychloroquine inhibits the translocation of monocyte NOX2 to the endosome in response to stimulants such as TNFα, IL-1β, and aPL [10▪▪]. This was accompanied by mitigation of aPL-induced, NOX2-mediated thrombus formation *in vivo* [10▪▪]. As the related drug chloroquine has been shown to antagonize NET release [21], further studies should continue to explore the intersection of hydroxychloroquine, activated monocytes/neutrophils, and APS.

Given its excellent safety profile in pregnancy [76], and its nearly standard-of-care application in

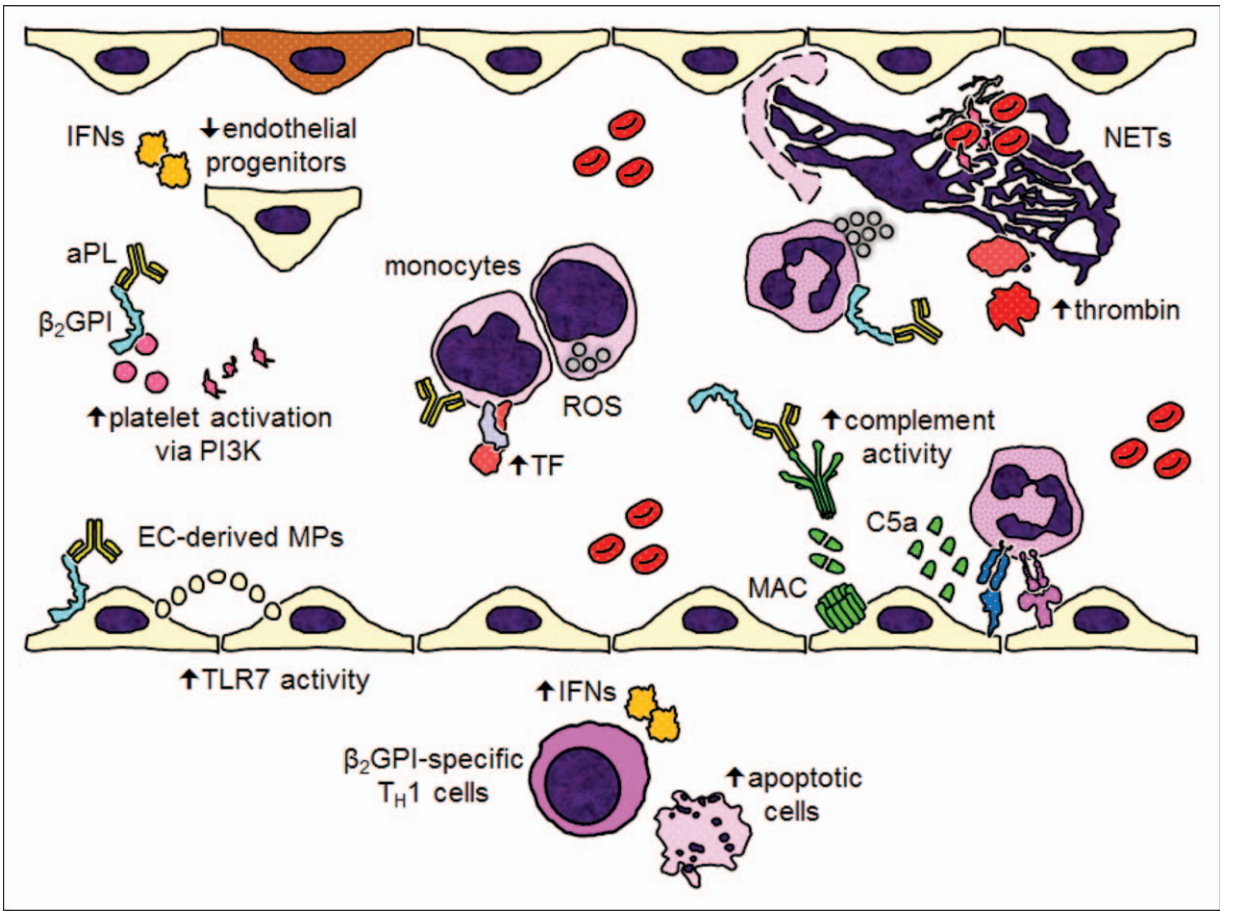

FIGURE 1. Recent mechanistic insights into the pathophysiology of antiphospholipid antibodies and antiphospholipid syndrome. Starting at the bottom of the figure and moving roughly clockwise: in the vessel wall of atherosclerotic plaques, beta-2 glycoprotein I-specific T_H1 cells trigger cell death and release interferons. Endothelial cells release vesicles (like microparticles) that activate Toll-like receptor 7 in other endothelial cells. Antiphospholipid antibody-mediated platelet activation relies on phosphoinositide 3-kinase. Type I interferons reduce the function of restorative circulating endothelial progenitors, Cofactor-independent antiphospholipid antibodies activate monocytes by endosomal reactive oxygen species, resulting in increased expression of tissue factor. In response to antiphospholipid antibodies, neutrophils release neutrophil extracellular traps, which help facilitate thrombin activation. Complement activation, especially through the classical pathway, leads to the assembly of the membrane attack complex on the endothelial surface, while also facilitating the recruitment and activation of inflammatory cells.

lupus pregnancies, hydroxychloroquine has been increasingly considered as adjuvant therapy in APS pregnancies. Indeed, recent retrospective studies have suggested a beneficial effect of hydroxychloroquine in APS pregnancies [7,17]. In a mouse model of obstetric APS, Bertolaccini *et al.* [15▪] recently demonstrated that hydroxychloroquine prevents fetal death and placental metabolic changes. Going further, they demonstrated that labeled aPL especially localize to the placenta and the developing fetal brain, and that hydroxychloroquine mitigates complement deposition at both sites (which correlated with lower levels of C3a and C5a in blood) [15▪]. Intriguingly, C3a and C5a were also reduced in the blood of APS patients after 6 months of hydroxychloroquine treatment [15▪].

CONCLUSION

Since its description in the 1980s, APS has been managed primarily with anticoagulant medications. These medications are not universally protective against subsequent thrombotic events and pregnancy loss and have little proven track record in treating 'noncriteria' manifestations of APS such as cytopenias and cardiac valvular disease. Basic science studies continue to refine the signaling pathways, activated cells, and noncellular effectors critical for APS pathogenesis (Fig. 1). In addition to a search for novel therapeutics, established medications such as statins and hydroxychloroquine are receiving increasing interest as adjuvant therapies. In the near future, we hope to see more well designed clinical trials with both mechanistic and clinical endpoints.

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Conflicts of interest

There are no conflicts of interest.

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Progress in the pathogenesis and treatment of cardiac manifestations of neonatal lupus

Peter Izmirly, Amit Saxena, and Jill P. Buyon

Purpose of review
To provide new insights into pathogenesis, prevention and management of cardiac manifestations of neonatal lupus (cardiac neonatal lupus) and issues pertinent to all anti-SSA/Ro positive individuals of childbearing age.

Recent findings
Antibody specificity with high risk for cardiac neonatal lupus remains elusive, but high titers of Ro60, Ro52 or Ro52p200 antibodies appear to be required. Varying antibody specificities to the p200 region of Ro52 can induce first-degree block in a rodent model. In consideration of the contribution of macrophages to inflammation and fibrosis in cardiac neonatal lupus, hydroxychloroquine (HCQ) is being considered as preventive therapy. Cord blood biomarkers support the association of fetal reactive inflammatory and fibrotic components with the development and morbidity of cardiac neonatal lupus. Data from U.S. and French registries do not provide evidence that the prompt use of fluorinated steroids in cases of isolated block significantly alters fetal/neonatal morbidity or mortality.

Summary
The search for a high-risk cardiac neonatal lupus antibody profile remains, but high-titer antibodies to Ro60 and R052 are a consistent finding, and this may guide the need for fetal echocardiographic surveillance. The uniform use of fluorinated steroids to prevent progression of cardiac neonatal lupus or reduce mortality does not appear justified. HCQ, based on diminishing an inflammatory component of cardiac neonatal lupus, is under consideration as a potential preventive approach.

Keywords
anti-SSA/Ro antibodies, congenital heart block, neonatal lupus

INTRODUCTION

Maternal autoimmunity is a significant environmental factor with the potential to irreversibly influence fetal and neonatal health. Although the relationship between systemic lupus erythematosus (SLE) and Sjogren's syndrome and congenital heart block (CHB) and neonatal skin rashes was described by 1960, the 'culprit' antibody reactivity to the SSA/Ro-SSB/La ribonucleoprotein complex was identified 20 years later [1]. Neonatal lupus was a term given to various fetal and neonatal manifestations associated with exposure to maternal anti-SSA/Ro-SSB/La antibodies [2]. With time came the remarkable realization that the mother's clinical disease was not the common denominator but rather this specific set of autoantibodies. In fact, bradycardia in a mid-to-late second trimester fetus is often the first clue to the presence of anti-SSA/Ro-SSB/La antibodies in a completely asymptomatic mother. The recent study from Stockholm County in Sweden reported only one of 20 cardiac neonatal lupus cases is born to a mother previously diagnosed with SLE [3]. The spectrum of cardiac manifestations of neonatal lupus (cardiac neonatal lupus) includes heart block (the most characteristic) and involvement beyond the atrioventricular node, myocarditis, dilated cardiomyopathies, valvular abnormalities and endocardial fibroelastosis. In this review, cardiac neonatal lupus will be used rather than CHB. The disease is rare with few population estimates. The recent Sweden study reported the incidence of anti-SSA/Ro autoantibody-related second and third-degree block to be 1 : 23,300 [3]. In mothers with the candidate autoantibodies, the disease occurs in 2% of pregnancies [4] and recurs in

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KEY POINTS

- There is not one singular epitope of Ro52p200 that is specific for the development of first-degree heart block in a murine model.
- Targeting downstream transcription factors and epigenetic modifications following Toll-like receptor 7/8 ligation in macrophages may be the mechanism of action of HCQ and provide rationale for its role in prevention of disease.
- On the basis of data from two large registries, treatment of advanced block with fluorinated steroids does not prevent further injury.

18% [5]. The mortality approaches 18%, and most children require lifetime pacing [6]. A major challenge in elucidating the mechanism of antibody injury relates to the fact that the target antigen is intracellular. Thus, accessibility to circulating maternal antibodies can be explained by either a cross-reactive cardiac myocyte surface antigen or cellular processes such as apoptosis that deliver the SSA/Ro or SSB/La antigens to the membrane surface. Surveillance of mothers at risk for cardiac neonatal lupus in an offspring relies on fetal echocardiograms. However, the low penetrance of disease, controversy over treatment of incomplete block if identified at all and irreversibility of complete block call into question the utility of such measurements. This review will cover bench-to-bedside studies from the recently published literature that provide insights into the pathogenesis and management of cardiac neonatal lupus.

UPDATES ON ANTIBODY SPECIFICITIES AND PATHOGENICITY

To date, two nonmutually exclusive hypotheses have been advanced to explain the molecular mechanism(s) by which anti-SSA/Ro-SSB/La antibodies to normally sequestered intracellular antigens initiate injury in the fetal heart. The first posits that the intracellular target antigens translocate to the surface of cardiomyocytes undergoing apoptosis during physiological remodeling and are bound by circulating maternal autoantibodies. The formation of pathogenic antibody-apoptotic cell immune complexes promotes proinflammatory and profibrotic responses [7–9]. The second hypothesis is based on molecular mimicry whereby antibodies cross-react with L-type calcium channels and cause dysregulation of calcium homeostasis [10–12]. Although several studies have attempted to identify specific epitopes within the SSA/Ro and SSB/La antigens that associate with cardiac neonatal lupus, most of these studies report epitopes common to the anti-SSA/Ro-SSB/La response regardless of fetal outcome. Importantly, different antibody subsets are identified depending on the immunoassay employed. Indeed, the sensitivity of peptide or recombinant protein ELISAs for anti-Ro60 antibodies is low and may result in false negatives [13,14].

Over the last decade, there has been a major focus on the antibody response against the p200 epitope, spanning Ro52 amino acids (aa) 200–239, as a candidate biomarker conferring an increased maternal risk for the development of cardiac neonatal lupus in an offspring [15,16]. The high prevalence of the p200 response in women giving birth to a child with cardiac neonatal lupus has been confirmed by several groups. However, there have been inconsistencies regarding its utility in high-risk assessment relative to the pregnancy exposure [17]. Consensus has not been reached as to whether this antibody response is also similarly observed in anti-SSA/Ro-exposed healthy children when all other maternal antibody reactivities to components of the SSA/Ro-SSB/La complex are equivalent. To overcome a limitation of most previous studies that prevalence and titer of maternal antibodies have not been measured during the time of fetal exposure, Reed *et al.* [18] evaluated umbilical cord blood and maternal serum during affected and unaffected pregnancies for reactivity to p200, full length Ro52, Ro60 and SSB/La. The frequencies of p200, Ro52, Ro60 and SSB/La autoantibodies were not significantly different between cardiac neonatal lupus and anti-SSA/Ro-exposed unaffected children. However, neonatal anti-Ro52 and Ro60 titers were highest in cardiac neonatal lupus neonates and their unaffected siblings compared to unaffected neonates without a cardiac neonatal lupus sibling. Although both maternal anti-Ro52 and p200 autoantibodies were less than 50% specific for cardiac neonatal lupus, anti-p200 was the least likely of the SSA/Ro autoantibodies to be false positive in mothers who have never had a cardiac neonatal lupus-affected child. Titers of anti-Ro52 and p200 did not differ during a cardiac neonatal lupus or unaffected pregnancy from the same mother. Thus, the utility of anti-p200 antibodies as a biomarker over standard commercial ELISAs (which report out positivity to SSA/Ro not specifically Ro52 or Ro60) to guide the level of fetal surveillance was not established.

Tonello *et al.* [19] reported on an Italian cohort of anti-SSA/Ro exposed pregnancies in 81 mothers (cardiac neonatal lupus in 16). As in the Reed's article, testing was done during the pregnancies. The prevalence of anti-p200 antibodies was

significantly higher in those mothers whose offspring developed cardiac neonatal lupus (advanced block) compared to those whose children were unaffected ($P = 0.03$). Likewise, combinations of anti-p200 with anti-Ro52 and anti-Ro60 antibodies were significantly more frequent in the women with fetuses developing cardiac neonatal lupus than in the controls. Women whose children had cardiac neonatal lupus had significantly higher mean anti-Ro52, anti-Ro60 and anti-p200 levels than the women whose children were unaffected ($P = 0.003$, $P = 0.0001$ and $P = 0.04$, respectively). However, a shortcoming emphasized in a dialogue reviewing this study was the fact that the investigators included mothers with low titer reactivities who would have been expected to be of lower risk [20].

Clearly, there is a need to better predict women at the greatest risk for the development of cardiac neonatal lupus in an offspring. To advance the field beyond what is already known, it would be important to enroll at the very least only women with high-titer antibodies during the pregnancy under study. However, it may be that even identifying the highest risk autoantibody profile is not sufficient and efforts to define fetal factors need greater emphasis.

Just as the clinical utility of identifying epitope specificity of the anti-Ro52 response has continued to be evaluated, likewise the pathogenicity of this response continues to be studied. The question persists: whether there is one single specific antibody profile underlying most cases of autoimmune-associated cardiac neonatal lupus, or whether there may be several antibody specificities and cross-targets involved. To this end, Hoxha *et al.* [21▪] have exploited a rodent model to define further the reactivity profile of anti-p200 antibodies. In brief, despite low-to-absent reactivity toward rat p200 and different binding profiles toward mutated rat peptides indicating recognition of different epitopes within Ro52p200, immunoglobulin (Ig)G purified from two mothers of children with cardiac neonatal lupus (advanced block) induced abnormalities in rat cardiac conduction. However, the abnormalities were restricted to prolongation of the PR interval and not second- or third-degree block. These findings support the hypothesis that several antibody specificities and cross-targets may exist and contribute to cardiac neonatal lupus in anti-Ro52 antibody-positive pregnancies. Thus, it is likely that there is not one single cardiac neonatal lupus inducing antibody specificity, but rather several different specificities that may act in an additive fashion to induce substantial damage in the fetal heart and lead to complete atrioventricular block. Unfortunately, as in prior studies using animal models, the cardiac phenotype remains mild. One explanation may be that levels of IgG crossing the placenta in rodents are insufficient to lead to full-blown inflammation and fibrosis of the murine fetal atrioventricular node. This consideration notwithstanding, even in humans it should be pointed out that placental transport at the 18–24-week vulnerable period is far less efficient than months later at term. Alternatively, essential fetal susceptibility factors may be absent in the mouse and rat strains studied thus far. In support of this possibility, it has been reported that fetal major histocompatibility complex modulates the penetrance of first-degree block in a rat model of cardiac neonatal lupus [22], and genetic variants modulating fetal cardiac function and/or inflammatory responses in the presence of maternal antibodies may amplify disease susceptibility and phenotype severity. At this time, a robust animal model that fulfills Koch's postulates and demonstrates advanced block with appropriate histologic correlates has not yet been developed.

Driven by the histologic features of cardiac neonatal lupus as demonstrated in autopsies of fetal hearts dying with the disease [23], Clancy *et al.* [24▪] have focused on an in-vitro model to recapitulate the underpinnings of the inflammatory infiltrate and subsequent fibrosis. On the basis of consistent demonstration of fibrosis of the atrioventricular node surrounded by macrophages and multinucleated giant cells, this group addressed macrophage signaling stimulated by ssRNA associated with the Ro60 protein and investigated the impact of antagonizing innate cell drivers such as toll-like receptor (TLR)7/8. Epigenetic modifications that affect transcription factors nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) and signal transducer and activator of transcription 1 were chosen to assess the phenotype of macrophages in which TLR7/8 was ligated following treatment with either anti-Ro60/Ro60/hY3 RNA immune complexes or transfection with hY3. On the basis of microarray, tumor necrosis factor alpha (TNF-α) and interleukin 6 were among the most highly upregulated genes in both stimulated conditions. This upregulation was inhibited by preincubation with hydroxychloroquine (HCQ), a drug which inhibits TLR ligation and is currently being studied to reduce the recurrence rate of cardiac neonatal lupus. In contrast, the resultant gene expression profile observed following stimulation with TNF-α or interferon alpha (IFN-α) (neither signal through TLR) was not inhibited by HCQ. Ligation of TLR7/8 resulted in increased histone methylation, a requirement for binding of NF-κB at certain promoters that was significantly decreased by HCQ. HCQ may act more as a preventive measure in downregulating the

initial production of IFN-α or TNF-α and may not directly affect the resultant autacoid stimulation reflected in TNF-α- and IFN-α-responsive genes. The potential benefit of antimalarials in the prevention of heart block in an anti-SSA/Ro antibody exposed offspring [25,26] may include, in part, a mechanism targeting TLR-dependent epigenetic modification.

To provide clues to the pathogenesis of cardiac neonatal lupus with translational implications for management, several candidate biomarkers in cases at risk for disease were evaluated [27▪]. The biomarkers were chosen based on their potential roles in inflammation, fibrosis and cardiac dysfunction: C-reactive protein (CRP), NT-pro-B-type natriuretic peptide (NT-proBNP), troponin I, matrix metalloproteinase (MMP)-2, urokinase plasminogen activator (uPA), urokinase plasminogen activator receptor (uPAR), plasminogen and vitamin D. On the basis of evaluation of 139 samples from the umbilical cord and 135 maternal samples, cord CRP, NT-proBNP, MMP-2, uPA, uPAR and plasminogen levels were higher in cardiac neonatal lupus-affected fetuses than in unaffected cases, independent of maternal rheumatic disease or medications taken during pregnancy. Maternal CRP and cord troponin I levels did not differ between the groups. Cord and maternal vitamin D levels were not significantly associated with cardiac neonatal lupus, but average maternal vitamin D level during pregnancy was positively associated with longer time to postnatal pacemaker placement. These data support the association of fetal reactive inflammatory and fibrotic components with development and morbidity of cardiac neonatal lupus independent of maternal risk factors. The authors suggest that following CRP and NT-proBNP levels after birth can potentially monitor severity and progression of cardiac neonatal lupus. MMP-2 and the uPA/uPAR/plasminogen cascade provide therapeutic targets to decrease fibrosis. Although decreased vitamin D did not associate with increased risk, given the positive influence on postnatal outcomes, maternal levels should be optimized.

APPROACH TO TREATMENT

Given the fetal bioavailability of fluorinated steroids and the presumed inflammatory response contributing to cardiac injury, these drugs have been considered in both the treatment and prevention of cardiac neonatal lupus. Although not uniformly effective, these drugs have been associated with reversal of first- and second-degree heart block [6,28–31]. As third-degree heart block has never been permanently reversed with any treatment, the utility of instituting fluorinated steroids with known side effects [30,32,33] has been questioned. Published data are limited and discordant regarding the efficacy of fluorinated steroids in reducing the mortality of cardiac neonatal lupus [28,34,35], which poses a therapeutic dilemma when isolated third-degree block is identified.

Leveraging data from a large registry of cardiac neonatal lupus cases, the efficacy of fluorinated steroids with regard to progression, mortality and need for pacemaker implantation was addressed [36▪▪]. In this retrospective study restricted to anti-SSA/Ro-exposed cases presenting with isolated advanced heart block in utero who received either fluorinated Steroids within 1 week of detection ($N = 71$) or no treatment ($N = 85$), the following outcomes were evaluated: development of endocardial fibroelastosis, dilated cardiomyopathy and/or hydrops fetalis; mortality; and pacemaker implantation. In Cox proportional hazards regression analyses, fluorinated steroids did not significantly prevent the development of disease beyond the atrioventricular node [adjusted hazard ratio = 0.90; 95% confidence interval (CI):0.43–1.85; $P = 0.77$], reduce mortality (hazard ratio = 1.63; 95% CI:0.43–6.14; $P = 0.47$) or forestall/prevent pacemaker implantation (hazard ratio = 0.87; 95% CI: 0.57–1.33; $P = 0.53$).

In aggregate, these data do not provide evidence that prompt fluorinated steroid use significantly alters fetal/neonatal morbidity or mortality. Variables that differed between treated and untreated groups included year of birth, which did not associate with extranodal disease, and HCQ use, which was so infrequent that it precluded meaningful analysis. Multivariable analyses revealed no identifiable maternal or fetal risk factor for progression of disease beyond the atrioventricular node. Consistent with previous reports, extranodal disease was significantly associated with mortality [6,28,37,38].

With regard to the efficacy of steroids to prevent the development of cardiac neonatal lupus, the Research Team for Surveillance of Autoantibody-Exposed Fetuses and Treatment of Neonatal Lupus Erythematosus, the Research Program of the Japan Ministry of Health, Labor and Welfare, performed a national survey on pregnancy of 635 mothers positive for anti-SSA/Ro antibodies. Cardiac neonatal lupus (advanced block) was detected in 16. In multivariate analysis, the use of corticosteroids before conception [odds ratio (OR): 4.28, $P = 0.04$] and high titer of anti-SSA/Ro antibodies (OR: 3.58, $P = 0.02$) were independent and significant risk factors for the development of cardiac neonatal lupus [39]. The use of corticosteroids (equivalent doses of prednisolone, at $\geq$ 10 mg/day) after conception

before 16 weeks of gestation was an independent protective factor against the development of cardiac neonatal lupus (OR: 0.16, $P = 0.03$). However, the use of continuous corticosteroids both before and after conception had no effect on the development of cardiac neonatal lupus. The difficulty in interpreting these results is that different preparations of steroids were used, making it challenging to sort out the effect of fluorinated steroids in particular. Moreover, there was only a small number of cases in which CHB developed ($N = 16$). Not unexpectedly, high titer of anti-SSA/Ro antibodies was an independent risk factor for cardiac neonatal lupus.

Levesque *et al.* [40▪▪] reported the results of a large retrospective French registry of 214 cases with cardiac neonatal lupus (advanced block). The use of fluorinated steroids was neither associated with survival nor with regression of second-degree CHB. The authors also leveraged this registry to address factors associated with mortality which in this cohort approached 16%. In agreement with previous publications [6,28], hydrops (hazard ratio = 12.4; 95% CI:4.7–32.7; $P < 0.001$) and prematurity (hazard ratio = 17.1; 95% CI:2.8–103.1; $P = 0.002$) were associated with fetal/neonatal mortality. During a median follow-up of 7 years (birth to 36 years), 148 of 187 children born alive (79.1%) had a pacemaker, 35 (18.8%) had dilated cardiomyopathy (DCM) and 22 (11.8%) died. In multivariate analysis, factors associated with child death were in utero DCM (hazard ratio = 6.37; 95% CI: 1.25–32.44; $P = 0.0157$), postnatal DCM (hazard ratio = 227.58; 95% CI: 24.33–2128.46; $P < 0.0001$) and pacemaker implantation (hazard ratio = 0.11; 95% CI: 0.02–0.51; $P = 0.0035$).

CONCLUSION

The search for a unique antibody profile that will predict the development of cardiac neonatal lupus remains elusive, but high titers of Ro60, Ro52 or Ro52p200 antibodies appear to be required. Varying antibody specificities to the p200 region of Ro52 can induce first-degree block in a rodent model. A robust animal model of cardiac neonatal lupus has yet to be developed. In consideration of the contribution of macrophages to the inflammation and fibrosis in cardiac neonatal lupus, HCQ is being considered as preventive therapy. Cord blood biomarkers support the association of fetal reactive inflammatory and fibrotic components with the development and morbidity of cardiac neonatal lupus. Data from two large registries do not support the use of fluorinated steroids in cases of isolated third-degree block as a means of preventing progressive injury.

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Conflicts of interest

There are no conflicts of interest.

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This is a very comprehensive description of an extremely well-characterized cohort. Several lessons are relevant: mortality is predicted by hydrops and prematuring, factors associated with child death were in utero and post natal dilated cardiomyopathy, pacemaker implantation was protective against child death and fluorinated steroids do not associate with a better outcome than not using fluorinated steroids.

Pregnancy and reproductive aspects of systemic lupus erythematosus

Laura Andreoli, Francesca Crisafulli, and Angela Tincani

Purpose of review
To discuss pregnancy and reproductive aspects in women with systemic lupus erythematosus (SLE) with particular focus on preconception counselling, maternal and foetal outcomes, safety and beneficial effects of drugs during pregnancy as well as contraception methods, assisted reproduction techniques and strategies for thromboembolism prophylaxis in patients with positive antiphospholipid antibodies.

Recent findings
Evidence-based recommendations for the management of family planning and women's health issues in SLE and/or APS have been developed by a multidisciplinary panel of experts. The primary aim of these recommendations is to provide a practical tool for facilitating physician–patient communication on reproductive issues. Points-to-consider and guidelines were also released on the use of antirheumatic drugs during pregnancy and lactation.

Summary
Women with SLE should be timely and periodically counselled on family planning. Preconception counselling and risk stratification (based on disease activity and serological profile) are key points for having successful pregnancies thanks to individualized treatments and close monitoring for maternal and foetal complications. Contraception and assisted reproduction techniques are feasible in women with SLE, provided that potential risks are minimized by individualized management and appropriate prophylaxis.

Keywords
assisted reproduction techniques, contraception, counselling, pregnancy, systemic lupus erythematosus

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multisystemic autoimmune disease affecting women predominantly in their childbearing age; therefore, it is essential to consider disease impact on pregnancy and reproductive aspects. Whereas in the past autoimmune diseases were considered to be an absolute contraindication to motherhood, today we know that pregnancy outcome in women affected by SLE has greatly improved thanks to a correct timing of pregnancy (discussed with the patients in a preconception counselling), a close monitoring throughout pregnancy and also in the postpartum period, a multidisciplinary management and an increased knowledge about the medications that can be used (in prevention or in case of disease's relapse) during pregnancy and breastfeeding [1▪,2▪▪,3▪▪,4].

In addition to pregnancy, contraceptive measures [5] and assisted reproduction technologies (ARTs) [6▪] are crucial topics to be addressed by the rheumatologist in the counselling about reproductive aspects.

This review will focus on preconception counselling and risk stratification in patients with SLE, maternal and foetal outcome [7▪▪,8], strategies for thromboembolism prophylaxis with particular reference to antiphospholipid antibodies (aPL), the use of antirheumatic drugs during pregnancy and breastfeeding, contraception, and assessment of fertility with special attention on feasibility of ARTs.

PREGNANCY

Ideally, rheumatologists should ask about family planning to each patient of childbearing age since the very first visit. The purpose is to give information about the correct timing of pregnancy in

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KEY POINTS

- Counselling about reproductive issues is crucial in women with SLE.
- Preconception counselling is essential for risk stratification and patient-tailored management in order to prevent adverse pregnancy outcomes.
- Most antirheumatic drugs are compatible with pregnancy and breastfeeding. Among these, the beneficial role of hydroxychloroquine during pregnancy should be emphasized.
- The intrauterine device can be offered to all patients (unless gynaecological contraindications). The use of hormonal contraceptive methods is feasible but must be weighed against the risk of thrombosis (in particular aPL profile).
- Assisted reproduction techniques are effective and generally safe in women with SLE with quiescent disease, provided that adequate antithrombotic prophylaxis is given and complications of ovarian stimulation are prevented (e.g., 'friendly ovarian stimulation').

Table 1. Risk factors to consider in women with SLE during preconception counselling

SLE-related risk factors	General risk factors
√ Active SLE in the previous 6–12 months or at conception	√ Maternal age
√ Active/history of Lupus Nephritis	√ Arterial hypertension
√ End-stage organ damage	√ Diabetes mellitus
√ Vascular thrombosis	√ Overweight or obesity
√ Previous adverse pregnancy outcome	√ Thyroid disease
√ Serological activity (C3, C4 levels, and antidsDNA titre)	√ Smoke and alcohol use
√ aPL profile (LA, aCL IgG/IgM, aβ2GPI IgG/IgM)	√ Immunization status (eg, rubella)
√ Anti-Ro/SSA, anti-La/SSB antibodies	

aB2GPI, anti β2GPI antibodies; aCL, anticardiolipin antibodies; AntidsDNA, antidouble stranded DNA; LA, lupus anticoagulant.
Adapted from Andreoli *et al.* [2▪▪].

relation to disease activity and about the compatibility of drugs needed for disease control.

Preconception counselling and pregnancy monitoring

Preconception counselling is crucial in women with SLE. The stratification of risk of adverse maternal and foetal outcome should be carried out by considering both disease-related and general maternal risk factors (Table 1) [2▪▪]. Once individual risks are established, the second step of the counselling is to set up preventive strategies and a patient-tailored monitoring plan. In addition to the routine ultrasonography screening (during the first trimester at 11–14 weeks of gestation and during the second trimester with Doppler at 20–24 weeks of gestation), patients with SLE should undergo supplementary surveillance in the third trimester, at monthly intervals, based on biometric and Doppler findings in order to diagnose an early or late Intra Uterine Growth Restriction and tailor the time of delivery [2▪▪,9–12]. Special monitoring is dedicated to women with Ro/SSA and/or La/SSB antibodies positivity; these patients should be informed about the risk of neonatal lupus and foetal dysrhythmia or myocarditis [13▪,14▪,15,16]. It is important to consider that the congenital heart block (CHB), the most feared complication associated with the presence of these antibodies, may occur from 0.7% to 2% in women with no history of foetal CHB, while the recurrence rate in a woman who already gave birth to a child affected by CHB is about 16% [17▪▪]. In the last case, it is recommended to perform foetal echocardiograms every week starting from week 16 of gestation. For women with positive anti-Ro/SSA and/or anti-La/SSB antibodies and no previous child affected by CHB, the current practice is to suggest a monitoring between 16 and 26 weeks of gestation, weekly or biweekly if possible. Despite its unproven benefit and cost-effectiveness, this intensive surveillance is safe and usually the patients are keen to accept it [17▪▪,18,19]. Patients with history of renal involvement should be encouraged to frequently monitor blood pressure and should perform 24-h urine protein analysis regularly [1▪]. The postpartum period can be critical for SLE flares; therefore, patients should be closely monitored and counselled about the possibility to breastfeed during the intake of antirheumatic drugs (Table 2).

It is therefore clear how important a multidisciplinary management is: rheumatologists, obstetricians, neonatologists and other specialized doctors should work together to ensure the best possible outcome for both the mother and the child.

Maternal and foetal outcome

The improvement in disease management and pregnancy monitoring have resulted in a significant decrease in pregnancy loss in SLE over the last 40 years (from an average of 43% in 1960–1965

Table 2. Compatibility of drugs with pregnancy and breastfeeding

Drug	Pregnancy	Breastfeeding
NSAIDs	YES During the first and the second trimesters	YES
Selective COX II inhibitors	AVOID (insufficient evidence)	Only Celecoxib
Prednisone	YES (at the lowest effective dose)	YES
Hydroxychloroquine	YES	YES
Azathioprine	YES (at doses up to 2 mg/kg/day)	YES
Methotrexate	AVOID (stop at least 3 months before pregnancy)	AVOID
Cyclophosphamide	AVOID (stop before conception; use justified only to treat life-threatening conditions during second and third trimesters)	AVOID (limited data)
Ciclosporin	YES (at the lowest effective dose)	YES
Tacrolimus	YES (at the lowest effective dose using trough levels)	YES
Mycophenolate mofetil	AVOID (stop 6 weeks before pregnancy)	AVOID (no data)
Immunoglobulins	YES	YES
Belimumab	LIMITED EVIDENCE, consider alternative medications	AVOID[a] (no data)
Rituximab	Can be used early in gestation in exceptional cases; in later stages of pregnancy there is risk of B cell depletion and other cytopenias in the neonate	AVOID[a] (no data)

[a]Theoretical possible use during lactation because monoclonal antibodies are large molecules and unlikely to be secreted in breast milk; if present in milk, monoclonal antibodies will be degraded in the neonatal gastrointestinal tract.
Adapted from Skorpen *et al.* [3▪▪]

to 17% in 2000–2003) and a trend toward a decrease in preterm births in SLE pregnancies [20]. However, in a recent population study the risk of stillbirths was found to be higher in patients with SLE compared to women from the general population [21].

In a meta-analysis including studies published between 2001 and 2016, maternal and foetal outcomes in pregnant women with SLE were compared to those of pregnant women without SLE [7▪▪]. In particular, a significant increase in caesarean section (RR: 1.85), preeclampsia (PE) (RR: 1.91), hypertension (RR: 1.99), spontaneous abortion (RR: 1.51), thromboembolic disease (RR: 11.29), and postpartum infection (RR: 4.35) were shown in pregnant women with SLE. Live births were significantly more frequent in women without SLE (RR: 1.38) while premature births were more common in women with SLE (RR: 3.05). In addition, 'small for gestational age' (SGA), birth weight less than 2500 g, necessity of neonatal intensive care unit, presence of congenital defects, and one minute APGAR score less than 7 were significantly higher among newborns of mothers with SLE [7▪▪].

This meta-analysis work is linked to several limitations, as reported by the authors. In fact, medications and treatment strategies used during pregnancy might not have been the same in all the hospitals; in addition, different SLE-clinical phenotypes have been included together in the analysis. A recent study focusing on early-onset PE (defined as PE registered at <34 weeks) showed a higher risk of this event in women with SLE than in the general obstetric population; this increase might be independent of the traditional risk factors like pregestational hypertension, antiphospholipid syndrome (APS), body mass index, or smoking [22▪].

An important aspect of pregnancy in patients with SLE is the risk of disease flares. Disease activity at conception and in the previous months is a predictor of both adverse pregnancy outcomes (APOs) and adverse maternal outcome (SLE flare): active disease during 6 months before conception is associated with an increase in the rate of pregnancy loss and active organ involvement in the same period predicts the same involvement during pregnancy [23,24,25▪,26]. A prospective multicentre study including seventy-one pregnancies in women

with lupus nephritis (LN) showed that, among the characteristics at baseline, high SLE disease activity index score, proteinuria, history of renal flares, arterial hypertension and active LN increased the probability of preterm delivery [27▪▪]. Moreover, the worst maternal outcomes have been observed in this group of patients [28,29]. Thus, patients with history of LN should deserve a particular management and follow up during pregnancy [1▪,30].

In a prospective, multicentre cohort study, baseline predictors of APOs (foetal or neonatal death; birth before 36 week because of placental insufficiency, hypertension or PE; SGA) included presence of lupus anticoagulant, antihypertensive use, Physician's Global Assessment score greater than 1, and thrombocytopenia [31▪▪,32]. Maternal flares, higher disease activity and smaller increase of C3 level later in pregnancy were also predictors of APOs [31▪▪]. Serological markers such as reduction of serum C3/C4 levels or increase of dsDNA titres are useful for the differentiation between disease exacerbation and PE [33,34].

Drug compatibility with pregnancy and adjunct treatment

One of the aims of the preconception counselling is to adjust treatment by switching to drugs compatible with pregnancy and adding drugs which are beneficial for pregnancy outcome [1▪]. Given that pregnancy should be planned in women with a stable remission of SLE, it is important to be able to maintain remission or to treat reactivation of disease during pregnancy, weighing the risk of potential side effects of drugs on the foetus with the negative impact of disease reactivation on the patient and her foetus. Recently, a European League Against Rheumatism task force has defined the points to consider for the use of antirheumatic drugs before pregnancy, and during pregnancy and lactation [3▪▪]. Compatibility with pregnancy and lactation is possible for antimalarials, azathioprine, ciclosporin, tacrolimus, intravenous immunoglobulin, and glucocorticoids (Table 2) [3▪▪,35▪▪,36▪▪]. Among these drugs, particular mention should be given to hydroxychloroquine (HCQ). A single randomized placebo-controlled and a few non-randomized studies highlighted that taking HCQ before and during pregnancy has a beneficial role in controlling SLE disease activity and preventing flares, thus it is absolutely indicated to continue it if already on treatment or to start it when pregnancy is planned [37–40]. Furthermore, HCQ may reduce the odds of CHB occurrence in foetus exposed to maternal Ro/SSA antibodies [13▪,41,42]. In a recent prospective multicentre study, the probability of having a small for gestational age baby was reduced by 85% in patients with LN who received HCQ therapy [27▪▪]. A beneficial role of HCQ has also been suggested for APS pregnancy [43,44,45▪] but there are still few data to recommend its routine use in these patients.

Methotrexate, mycophenolate mofetil, and cyclophosphamide require discontinuation before conception due to proven teratogenicity (Table 2). Insufficient documentation implies the discontinuation of rituximab, belimumab, and other biologic drugs before a planned pregnancy [3▪▪,46].

In pregnant patients at high risk of PE without autoimmune disease, the introduction of low-dose aspirin (LDA) before the 16 weeks of gestation has resulted in a reduction of the risk of PE, foetal growth restriction, preterm birth and perinatal death [47,48]. Accordingly, pregnant women with SLE at risk of PE, in particular those with LN and aPL positivity or APS, should start LDA preconceptionally or no later than 16 week of pregnancy [2▪▪]. In patients with APS the association of LDA and low molecular weight heparin (LMWH) is recommended [49,50]. Among patients with the positivity of aPL but without a formal diagnosis of APS, this association is recommended in selected cases such as older maternal age, high-risk aPL profile (lupus anticoagulant, multiple aPL, moderate to high titre of aPL) and during assisted reproduction techniques. Conversely, patients with a low-risk aPL profile could be candidate to a less-aggressive approach [1▪,2▪▪,51] (Table 3).

As in the general obstetric population, a supplementation with folic acid, calcium, and vitamin D is recommended [2▪▪].

CONTRACEPTION

A major concern is to avoid pregnancy during disease flares or during the intake of potentially teratogenic drugs. Nevertheless, the use of hormonal contraceptive methods may favour an increased risk of disease reactivation and thrombotic events. [2▪▪] For these reasons, advice about contraception is crucial for these women; however, the patients have been reporting gaps in the provision of such counselling [52,53].

Currently available contraceptives include barrier methods, oral hormonal contraceptives, and intrauterine devices (IUDs). Oral hormonal contraceptives have been discourage in past. Nevertheless, a recent review and two randomized controlled trials have demonstrated that combined oestrogen-progestin and progestin-only pills are safe for inactive and stable disease, in the absence of aPL antibodies [5,54,55]. Major concerns are about

Table 3. Adjunct therapy during pregnancy in relation to thrombotic risk factors

Characteristics of the patient	Medication
All SLE patients	LDA
History of pregnancy failure despite treatment with LDA	LDA + LMWH at prophylactic dose[a]
High aPL risk profile	
aPL and additional thrombotic risk factors	
APS with history of early recurrent miscarriages, fetal death, PE	
History of pregnancy failure despite treatment with prophylactic dose of heparin	LDA + LMWH at full anticoagulant dose
High aPL risk profile and additional thrombotic risk factors	
History of venous or arterial thrombosis	

LDA low-dose aspirin; SLE, systemic lupus erythematosus.
[a]In patients with positivity of aPL, LMWH should be given also during puerperium (up to 6 weeks after delivery).
Adapted from Andreoli *et al.* [2▪▪].

women with aPL positivity or definite APS, in whom oestrogens containing preparations are contraindicated for the increased risk of thromboembolism; in these patients progestin-only preparations could be considered, although this risk is not absent [1▪]. IUDs can be offered to all patients unless gynaecological contraindications [55]. Copper IUD has no systemic side effects but often increases dysmenorrhoea and menstrual bleeding, while levonorgestrel IUD has the advantage of reducing dysmenorrhoea and menstrual bleeding with a not significantly increased risk of thrombosis [56]. Barrier or natural methods are the least effective ones [56].

In conclusion, decisions regarding any contraceptive method in patients with SLE or APS must take into account not only the prevention of unintended pregnancy but also the efficacy, the ease of use and the risks of the method.

FERTILITY AND ASSISTED REPRODUCTION TECHNIQUES

SLE patients have fewer children than other women. Recently, a longitudinal observational study highlighted that, among patients with SLE interested in having children, 64% had fewer children than originally planned [57]. This is mainly due to the higher rate of foetal losses and it has not been associated with an increased rate of primary infertility, as assessed by the determination of hormonal levels or by the antral follicle count in ultrasound [58–61]. In addition to this, some alkylating agents such as cyclophosphamide can lead to premature ovarian failure, which is age and dosage dependent [62,63]. Another issue to be consider is that women with SLE often are allowed to plan a pregnancy later than women in general population, with a physiological decline of fertility [64].

Consequently, in order to overcome the difficulties for successful pregnancies, the number of women with SLE opting for ARTs is constantly growing. ARTs, which include ovulation induction therapy and in-vitro fertilization, require ovarian stimulation that is administered in order to obtain a multiple follicular growth [65]. Current stimulation protocols can increase the risk of lupus flares [66], thrombotic events, and ovarian hyperstimulation syndrome [67]. Therefore, it is essential to individualize ARTs procedures to the patient's profile.

ARTs are generally safe if the patient has quiescent disease [6▪]. Friendly ovarian stimulation, single embryo transfer, antithrombotic prophylaxis, and use of natural oestrogen or progestin through a nonoral route may constitute the safest approach [68,69▪].

Active SLE, poorly controlled arterial hypertension, advanced renal disease, severe valvulopathy or heart disease, and major previous thrombotic events are all situations for discouraging ARTs, especially due to the high risk of complications for both mother and foetus during pregnancy and puerperium [66].

In women with positive aPL undergoning ovarian stimulation, some general measures for prophylaxis can be suggested. The type and dosage of antithrombotic treatment should be recommended as during pregnancy according to the individual risk profile. LDA should be stopped three days before eggs retrieval and resume the following day, while LMWH should be stopped 12 h prior the procedure and resumed the very same day as long as there is no bleeding [2▪▪].

Regarding efficacy, pregnancy rate in SLE patients is comparable with that of the general population (up to 30%) [70]. aPL positivity is no predictor of ARTs failure [71].

CONCLUSION

Reproductive aspects including pregnancy, contraception, and feasibility of ARTs are a major concern in patients with SLE. Thanks to increased awareness of risk factors, predictive biomarkers of disease reactivation and drugs that can be used in pregnancy, women with SLE can fulfil their family planning. Preconception counselling and patient follow-up during gestation are crucial in order to improve patient and foetal outcomes by the assessment of risk factors and the early recognition of disease flares or pregnancy complications. Counselling about contraception should be given to the patients in order to weigh the risk of unintended pregnancy against the risk of thrombosis or disease reactivation. Although SLE itself is not a cause of infertility, these women may have difficulty in conceiving, especially if alkylating agents have been used. It is important to know that ARTs in these women are effective and generally safe if the patient has quiescent disease and is on appropriate antithrombotic treatment if aPL positive.

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There are no conflicts of interest.

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Socioeconomic consequences of systemic lupus erythematosus

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Purpose of review
The present review addresses recent literature investigating the socioeconomic consequences of systemic lupus erythematosus (SLE). We highlight the latest updates on health disparities affecting the SLE population, the direct and indirect economic costs of the disease, and less quantifiable costs such as reduced health-related quality of life (HRQoL).

Recent findings
Health disparities continue to exist among socially disadvantaged populations, including African Americans, Hispanics, and patients with decreased educational attainment and in poverty. Direct and indirect costs are substantial. Recent work provides updated cost estimates for patients with SLE outside of North America, including those in developing countries. Previous research has largely focused on costs of the general SLE population and those with renal manifestations or active SLE, whereas recent research addresses special populations such as hospitalized and pregnant patients and glucocorticoid users. Patients with SLE and their caregivers experience a substantially reduced HRQoL.

Summary
SLE is a costly disease that disproportionately affects disadvantaged populations. Future economic studies should measure not only direct costs, but also incorporate indirect costs and the HRQoL of both patients with SLE and their caregivers. All these components are essential to provide a comprehensive assessment of the socioeconomic consequences of SLE and an appreciation of the potential impact of novel therapies.

Keywords
direct costs, health-related quality of life, indirect costs, socioeconomic, systemic lupus erythematosus

INTRODUCTION

Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease, characterized by multiorgan involvement. Severity ranges from cutaneous manifestations to life-threatening organ failure. SLE particularly impacts patients of lower socioeconomic status, with increased disease prevalence and severity. Young women in their peak reproductive and employable years are predominantly affected, causing considerable social and economic impact.

Disease costs can be described as direct, indirect, or intangible. Direct economic costs include quantifiable expenditures related to the prevention, diagnosis, and treatment of the disease. Indirect costs represent those associated with decreased labor and nonlabor market activities (such as childcare and household work). Intangible costs, represented by decreased health-related quality of life (HRQoL), are more difficult to quantify, but comprise much of the illness burden of SLE.

The present review summarizes the latest work describing the socioeconomic impact of SLE, focusing on the causes and consequences of health disparity and direct, indirect, and intangible costs.

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KEY POINTS

- Both socioeconomic and genetic factors underlie health disparities in SLE.
- Direct costs continue to be substantial and are driven by disease activity and disease damage.
- Recent work continues to define costs in special SLE populations including hospitalized and pregnant patients as well as glucocorticoid users.
- The indirect and intangible costs of SLE, and the burden to informal SLE caregivers, are enormous and should be considered in future economic studies.

Understanding the socioeconomic factors contributing to and the magnitude of the burden of SLE is essential in guiding future efforts to reduce disease impact both to society and the individual patient.

Health disparity and systemic lupus erythematosus

A large body of evidence, recently reviewed in ref. [1▪], describes the health disparity or disproportionate burden of SLE in disadvantaged populations. Health disparity can be attributed to a variety of factors. Genetic, environmental, and socioeconomic factors, including educational attainment, financial resources, healthcare access, and social support can influence disease prevalence, severity, and outcome. Much of the literature has focused on the African American population. African Americans are disproportionately affected by SLE, having increased disease prevalence and severity, damage accrual, renal involvement, and mortality and poorer HRQoL, reviewed in ref. [2]. Other populations substantially affected by SLE include the American Hispanic, Asian, and Aboriginal populations, reviewed in ref. [1▪].

It is difficult to distinguish the role of increased genetic susceptibility to SLE from contributing socioeconomic factors in minority populations. African Americans had an increased risk of death compared to non-African Americans in a retrospective cohort study of 12 352 patients with lupus nephritis induced end-stage renal disease [adjusted hazard ratio (HR) 1.18; 95% confidence interval (CI), 1.11–1.25]. However, adjusting for area-level median household income attenuated this risk (adjusted HR 1.09; 95% CI, 1.02–1.15), suggesting that socioeconomic status is a major determinant of ethnic disparity in the outcome of SLE-related end-stage renal disease [3].

A recent comparison of 114 Hispanic patients from Texas in the Lupus in Minorities: Nature Versus Nurture (LUMINA) cohort with 619 Latin American Mestizo patients in the Grupo Latino Americano de Estudio de Lupus (GLADEL) cohort showed increased damage accrual, as measured by the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index [SDI; relative risk (RR) 1.33; 95% CI, 1.12–1.58], and mortality (HR 2.37; 95% CI, 1.10–5.15) in the Hispanic patients, despite similar genetic backgrounds [4]. This finding is again suggestive of the influence of socioeconomic factors on disease outcome, and therefore modifiable factors may attenuate some of the risk associated with ethnicity.

Both African American and Hispanic patients with SLE have worse pregnancy outcomes than Caucasian patients with SLE. A multivariate analysis of 13 553 SLE deliveries from an American hospital discharge database demonstrated increased preterm labor [African American vs. Caucasian odds ratio (OR) 1.59; 95% CI, 1.41–1.79, Hispanic vs. Caucasian OR 1.51; 95% CI, 1.32–1.73], preeclampsia (African American vs. Caucasian OR 1.16; 95% CI, 1.02–1.32, Hispanic vs. Caucasian OR 1.44; 95% CI, 1.26–1.65), and intrauterine growth restriction (African American vs. Caucasian OR 1.50; 95% CI, 1.26–1.79, Hispanic vs. Caucasian OR 1.60; 95% CI, 1.32–1.94) [5▪▪]. Additionally, there was a significant increase in stillbirth rate in the African American patient group compared to Caucasians (OR 1.61; 95% CI, 1.15–2.25). Acute medical complications including renal failure, pneumonia, and transfusion were also increased in African American and Hispanic patients at the time of delivery. The cost of delivery was correspondingly higher in African American (19% increased) and Hispanic patients (42% increased) compared to Caucasians. The causes of the ethnic disparities were not identified in this study. Conception planning and pregnancy may represent a particularly critical juncture for SLE monitoring and treatment in minority populations.

Educational attainment is known to affect SLE disease outcome. The Canadian 1000 Faces of Lupus investigators assessed whether education, as a surrogate for socioeconomic status, influenced work disability, disease activity, and organ damage in a publicly funded healthcare system in a prevalent population of 562 patients. Low education (not completing high school) as opposed to education beyond high school was associated with double the likelihood of work disability (30 vs. 14%, $P = 0.0001$), and increased disease activity as measured by the SLE Disease Activity Index (SLEDAI), but not increased damage [6▪]. The influence of education on SLE was

also investigated by the Chinese SLE Treatment and Research Group (CSTAR) on 904 therapy-naive patients in China. Multivariate regression analysis showed that lower education was associated with higher disease activity as measured by SLEDAI (β coefficient = −0.122; $P = 0.001$) [7▪].

Recent literature has started to clarify the association between income and disease outcome in emerging countries. A cross-sectional study of 143 Mexican patients with SLE demonstrated that lower monthly household income was significantly associated with organ damage (SDI $\geq$ 1; OR 4.6; 95% CI, 1.3–16.1) but not disease activity [8].

Providing access to specialist care may mitigate some of the effect of income on SLE outcome. Provision of healthcare in Puerto Rico occurs both privately and publicly, but publicly funded care is available only to patients with reduced income. A cross-sectional study of 98 Puerto Rican patients with SLE demonstrated that patients with publicly funded care had improved patient-reported outcomes compared to private patients, despite being more likely to have renal disease [9▪]. The authors suggest that this apparent discrepancy in association between socioeconomic status and patient-reported outcomes may be related to enhanced access to specialty clinics for publicly funded patients.

Social support is another modifiable component of socioeconomic status. Patients with SLE who rated their healthcare providers in the lowest quartile for patient–physician communication had increased damage in the subsequent 2 years as measured by at least a two-point increase in Brief Index of Lupus Damage (BILD) score (adjusted OR 2.35; 95% CI, 1.25–4.39). Patients who felt their care coordination was poor also accrued more disease damage (adjusted OR 2.20; 95% CI, 1.12–4.34). Thus, improving healthcare provider–patient relationships may favourably influence SLE outcomes [10▪].

One of the few papers to specifically focus on health disparities experienced by youth with SLE/mixed connective tissue disease interviewed 16 patients and reported that those who adapted poorly to their disease had lower socioeconomic status, HRQoL, and psychosocial functioning, and increased disease morbidity [11].

Direct economic costs of systemic lupus erythematosus

Despite the fairly low prevalence of SLE, some patients, particularly those with advanced disease and organ failure, can accrue considerable costs, making the societal impact quite substantial. Direct costs include healthcare visits, hospitalizations, medications, diagnostic and therapeutic laboratory and imaging procedures, and renal replacement therapies. Previous literature estimates annual costs to be $34 146 for the general SLE population, $73 306 for patients with lupus nephritis, and between $13 869 and $56 882 for patients with severe or active SLE [1▪].

Recent literature provides updated cost of illness estimates for populations outside of Canada and the United States. A South Korean cohort study of 749 prevalent patients revealed that mean annual direct costs were $3692 (SD $5909) with predictors of increased cost including higher disease activity (as measured by the SLEDAI-2K) and damage per the SDI, and renal and hematologic involvement per ACR classification criteria [12].

Other recent costing updates include a cohort study of over 1000 prevalent patients with SLE from Sweden. This study reported an annual total cost of $36 138 (SD $ 49 473), with direct costs of $11 033 (SD $30 704), including outpatient visits, inpatient days, and medications. These costs were likely underestimated, as primary care visits were not included in the analysis. Disease activity (SLEDAI-2K > 3) was associated with a 50% increase in direct and indirect costs. Costs were also increased with advancing age and renal, neuropsychiatric, and musculoskeletal organ damage [13].

A chart review of over 200 patients in Greece demonstrated annual direct costs that were threefold higher in patients with severe disease [defined as active involvement of renal, neurological, cardiovascular, or respiratory domains by the modified British Isles Lupus Assessment Group (BILAG) disease activity index and requiring a prednisone equivalent >7.5 mg/day and/or immunosuppressants], as opposed to nonsevere patients [mean annual direct medical cost $5181 (SD $7873) vs. $1697 (SD $2831)] [14]. Cost predictors included an SDI score >0, disease flares, renal involvement, and advancing age.

Recent literature is addressing, for the first time, costs that occur in specific lupus populations, including those who are hospitalized, pregnant, or on glucocorticoids. Anandarajah *et al*. examined the reasons precipitating admission to a New York hospital. Infection was the most common responsible diagnosis for both admission and readmission. The average length of stay was 8.5 days with mean hospitalization direct costs per admission of $ 20 934 [15▪]. Twenty-four percent of outpatients with SLE required admission annually. Forty-five percent of all admissions were readmissions, the majority of which occurred within a month of the initial hospitalization. Given the preponderance of infectious admissions, prompt access to outpatient care and

vaccination optimization are suggested strategies to reduce SLE admissions [15▪].

Direct costs have recently been estimated for pregnant SLE patients. A U.S. healthcare claims database demonstrated increased direct costs in 1721 pregnant women with SLE compared to 8605 pregnant women without SLE. Mean costs were $22 821 (SD $25 929) in SLE patients for the duration of their pregnancy and postpartum period, versus $12 182 (SD $11 267) for pregnant women without SLE. Medication, outpatient, and inpatient costs were all increased in patients with SLE [16].

Increased healthcare utilization and costs occur with advancing glucocorticoid use. A U.S. insurance claims database showed annual total costs of $22 849 (SD $49 379) among all patients with SLE, whereas patients with no glucocorticoid exposure in the preceding year had total costs of $17 148 (SD $39 226) and high-dose users had costs of $48 128 (SD $80 922) [17▪]. Low-dose glucocorticoid users had reduced costs if also prescribed glucocorticoid-sparing agents. As the study was observational and lacking randomization, it was unable to address whether the increased costs associated with glucocorticoid use reflect increased disease severity and are therefore confounded by indication, or whether costs are increased because of glucocorticoid-related adverse events.

A 2016 study of 1611 incident SLE patients identified via a U.S. claims database again demonstrated that those patients receiving glucocorticoid monotherapy had the highest costs compared to patients receiving other medication classes. Moderate corticosteroid users had direct costs of $96 846 (SD $165 147) over 4 years as opposed to $53 639 (SD $108 966) for all patients with SLE. Patients receiving persistent heavy hydroxychloroquine monotherapy had lower direct costs [$39 515 (SD $58 336)] than either all other patients with SLE, or minimally treated patients [$48 012 (SD $116 188)] [18▪].

Indirect costs of systemic lupus erythematosus

Indirect SLE costs have been estimated at $1287–$20 603 per year, reviewed in ref. [1▪].

A recent Swedish cohort study, mentioned previously, demonstrated that 70% of total costs are indirect. This study considered work absence, but not lost productivity in nonlabor activities such as childcare. Higher indirect costs were associated with female, advancing age, and neuropsychiatric and renal organ damage [13].

A recent study reported indirect costs incurred by caregivers of patients with SLE, a group not typically reflected in economic analyses. Al Salwah *et al.* [19▪▪] surveyed over 250 SLE caregivers and found that most are the intimate partners of patients with SLE. Seventy-five percent of caregivers assisted the patient financially and many reported negative effects on work such as leaving paid employment, working less hours, decreasing work responsibility, earning less income, and experiencing increased stress at work. Future studies which quantify indirect costs to not only SLE patients, but also their family members, will better capture the true economic burden of SLE.

Intangible costs of systemic lupus erythematosus

Health-related quality of life refers to the effect of disease on a patient's perception of their overall wellbeing, including physical, emotional, and social domains.

Factors associated with reduced HRQoL in 467 international childhood-onset SLE patients included female sex, increased disease activity and damage, non-Caucasian ethnicity, and use of cyclophosphamide and/or rituximab [20▪]. Adult patients also reported decreased HRQoL with increased damage occurrence [21].

In the SLE caregivers' study described above, caregivers also reported several psychosocial stressors, including decreased socialization and engagement with personal interests, as well as increased anxiety and stress. Burnout was present in over a third of caregivers. Almost 60% of caregivers felt their burden would be eased with additional advice regarding how to respond to medical problems, and more focus on the emotional impact of caring for patients with SLE. Thus, a concerted effort by healthcare providers to engage caregivers in medical appointments and refer them for psychosocial supports is needed [19▪▪]. In a survey of 162 patients with SLE [22▪], increased self-efficacy was associated with improved HRQoL, again underscoring the importance of psychosocial supports in the SLE population.

CONCLUSION

The latest literature on the socioeconomic consequences of SLE continues to demonstrate health disparities in disadvantaged populations, including African Americans, Hispanics, and patients with decreased education, income, and healthcare access. Identifying populations who are at highest risk for severe SLE is essential to ensure that appropriate resources are allocated to the most vulnerable.

It is difficult to compare direct costs across studies as estimates are heavily influenced by the study design and are often incomplete. Many SLE

costing studies utilize insurance claims databases that have the advantage of capturing large populations, but they cannot stratify patients with SLE according to disease severity and damage and they exclude the uninsured. Although cohort studies are expensive, they provide much more diagnostic certainty and data regarding disease severity, which is particularly important in a disease as phenotypically diverse as SLE. Much of the focus of costing is on direct costs. For a disease that disproportionately affects young women in their peak reproductive and educational/economic years, the indirect costs of lost productivity are enormous and need to be incorporated into economic analysis. Future studies should also endeavor to include economic costs for informal SLE caregivers, whom are typically intimate partners. This will provide a more accurate representation of actual economic cost to families. Detailed economic studies are necessary to demonstrate that the anticipated costs associated with the novel emerging therapies are likely to be commensurate with their benefits.

Intangible costs, as represented by decreased HRQoL, are arguably the most significant to patients. Drivers of HRQoL include damage accrual, and thus prompt diagnosis, minimization of glucocorticoid use, and aggressive treatment of SLE is predicted to improve HRQoL. Insight into the SLE illness experience allows for a therapeutic relationship between the healthcare team and the patient, and provides a compelling compassionate argument for funding of allied health supports such as physiotherapy, social work, and psychology to mitigate the impact of illness.

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Conflicts of interest

There are no conflicts of interest.

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Musculoskeletal manifestations of systemic lupus erythematosus

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Purpose of review
Imaging studies suggest potential changes to the classification and assessment of inflammatory musculoskeletal lupus. This is important because of the burden of disease but the potential for new targeted therapies.

Recent findings
Using our current classification and treatment, musculoskeletal symptoms continue to impact significantly on quality of life and work disability. Ultrasound and MRI studies suggested that new approaches to the diagnosis, classification, and evaluation of these symptoms are needed. Many patients with pain but no synovitis have ultrasound-proven joint and tendon inflammation but would not qualify for clinical trials or score highly on disease activity instruments. MRI studies show that erosions are more common than previously thought and may have a different pathogenesis than RA. Immunology studies suggest differences from other autoimmune synovitis, with a complex role for type I interferons. A wide range of biologic therapies appear more consistently effective for arthritis than some other manifestations.

Summary
Changes to the selection of patients for therapy and stratification using musculoskeletal imaging may offer new approaches to clinical trials and the routine care of systemic lupus erythematosus patients with inflammatory musculoskeletal symptoms. Outcomes may thereby be improved using existing therapies. There are significant knowledge gaps that must be addressed to achieve these potential improved outcomes.

Keywords
arthritis, biological therapy, MRI, systemic lupus erythematosus, ultrasonography

INTRODUCTION

Musculoskeletal manifestations are among the most common features of systemic lupus erythematosus (SLE) both in initial diagnosis and in long-term management. They are crucial to overall patient outcome as well as the development of new therapeutics. This review concentrates on impact, classification, assessment, and treatment of inflammatory musculoskeletal manifestations.

IMPACT OF MUSCULOSKELETAL MANIFESTATIONS OF SYSTEMIC LUPUS ERYTHEMATOSUS

Musculoskeletal manifestations of SLE are the first presenting symptom in up to 50% of SLE patients and affect up to 95% during the clinical course [1–3]. Although other manifestations may be more important in causing organ failure and early mortality, musculoskeletal manifestations are the key determinant of impact of disease for a larger group of patients. Apart from fatigue, the most frequent symptoms reported by 324 SLE patients in answer to the question 'What SLE-related symptoms have you experienced as most difficult during your disease?' were pain (50%) and musculoskeletal (46%). Further, these symptoms were most strongly related to reduced health-related quality of life [4]. In systematic review, 47% of SLE patients were employed and 34% had work disability [5]. Of individual features of SLE disease activity, arthralgia was the only one to be significantly associated with work disability, with

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KEY POINTS

- Musculoskeletal manifestations are major contributor to poor quality of life and work instability despite current therapy.
- Clinical assessment underestimates level of joint and tendon inflammation compared to ultrasonography and MRI with implications for patient selection for therapy and evaluation of response.
- Erosions are more common in SLE than previously thought and may have a different pathogenesis to rheumatoid arthritis.
- Type I interferons may have a complex role in synovitis in SLE and some RA patients yet are a promising therapeutic target.
- A wide range of biologic therapies appear consistently effective for musculoskeletal disease.

odds ratio (OR) 2.41 [95% confidence interval (CI) 1.53–3.79] [6]. By comparison, overall disease activity measured by systemic lupus erythematosus disease activity index (SLEDAI) had no association with work disability, and by SLAMM had only a modest association (OR 1.12, 95% CI 1.03–1.21). Only age, household income, and fibromyalgia were more strongly associated with work disability. In longitudinal follow-up, 34% of patients with musculoskeletal manifestations stopped working after median 4 years [7]. Arthralgia is, therefore, one of the most important modifiable factors in disability and participation in SLE patients.

Musculoskeletal manifestations of SLE are frequently treated with glucocorticoids and NSAIDs, both of which may increase the rate of long-term cardiovascular complications. A recent French study found that cardiovascular disease is the greatest cause of mortality in SLE [8], and in a 2016 systematic review cardiovascular diseases were most strongly associated with increased standardized mortality ratios in SLE after renal disease and infection [9]. Interestingly, cluster analysis of systemic lupus international collaborating clinics/American college of rheumatology damage index domains found clusters defined by low damage, musculoskeletal damage, and cardiovascular damage, with death rates of 3.7, 10.8, and 20.5%, respectively [10▪].

Regarding therapeutics: the majority of nonrenal lupus patients qualify for clinical trials and biologic therapy because of disease activity in skin and musculoskeletal systems. For example, in the ILLUMINATE-1 phase III trial of tabalumab, 78.8–83.5% of patients had activity in the musculoskeletal system in each arm. Only skin disease was more common, and the proportions of patients with activity in other organs was far smaller: the next largest clinical manifestation was haematologic – approximately 11% of patients [11]. In total, 31% of all renal and nonrenal SLE patients treated with a biologic in the UK-based British Isles Lupus Assessment Group Index (BILAG) biologics registry received it for musculoskeletal disease [12].

CLASSIFICATION OF MUSCULOSKELETAL SYSTEMIC LUPUS ERYTHEMATOSUS

Many previous reviews, have focused on two clinically distinctive phenotypes of lupus arthritis: Jaccoud's arthropathy and Rhupus. Jaccoud's arthropathy was first described as a nonerosive arthropathy with reversible deformities in association with rheumatic fever. An identical phenotype was noted in patients with SLE in 1975 [13], although it may also occur in other connective tissue diseases. Although, this appearance is now frequently cited in textbooks as being highly characteristic of SLE, it is in fact quite uncommon. Estimates of prevalence from the modern era of investigation of SLE range from 2.8 to 3.5% [14,15].

Approximately 5% of SLE patients are estimated to present with 'Rhupus'. These patients meet criteria for both Rheumatoid arthritis (RA) and SLE, have an erosive arthritis with identical radiographic appearance to RA, and has also been associated with rheumatoid factor and anticitrullinated peptide antibodies, suggesting common pathogenesis and genetics to more typical RA [16,17]. A recent study compared features of lupus arthritis in children and adults. Although chronic polyarthritis arthritis tended to be more common and severe in children, rates of Jaccoud's and Rhupus did not differ [18▪].

The vast majority of patients with lupus arthritis are, therefore, often described a nondeforming nonerosive (NDNE) arthritis. Although these patients all have similar inflammatory features to other inflammatory arthritis, such as symmetrical small joint distribution and morning stiffness, clinically detectable synovitis is present in a minority of cases. Disease activity indices and inclusion criteria for clinical trials have tended to focus on the subset of patients clinical synovitis (discussed further below).

Recent investigative techniques have led to the proposal of alternative classifications that would change approaches to assessment and treatment [19,20].

Subclinical synovitis

One of the most important, of recent insights, has been the observation from ultrasound studies that

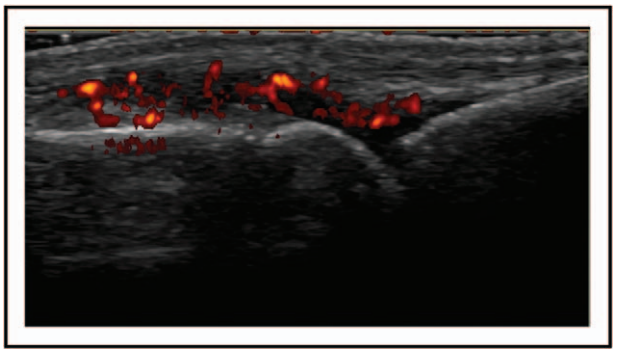

FIGURE 1. High resolution ultrasound image of an MCP joint of a patient with systemic lupus erythematosus. This patient had pain in small joints with morning stiffness and symmetry, but no clinical swelling, warmth, or effusion. The BILAG musculoskeletal domain score was, therefore, C. The ultrasound confirms greyscale as well as power Doppler synovitis – abnormalities considered to be definite active inflammation in other inflammatory arthropathies. MCP, metacarpopharngeal.

large numbers of lupus patients with arthralgia, despite the lower rates of synovitis compared to other inflammatory arthritides (Fig. 1). This is crucial for clinical practice and trials because existing clinical disease activity instruments are all heavily weighted by the presence of synovitis (see below). Our group recently published a systematic review of these studies [21▪▪]. Although nine studies including 459 patients all agreed with the existence of subclinical synovitis, there were methodological considerations that needed addressing before these results could be applied to clinical practice. Rates of Ultrasound-detected synovitis reported in these studies vary widely. For example, rates of power Doppler abnormality ranged from 10 to 82%. One reason, for this may be the inclusion of variable numbers of Rhupus patients in most of the studies. Second, although most studies reported using outcome measures in rheumatoid arthritis clinical trials (OMERACT) definitions for ultrasound abnormality, few of them actually reported levels of abnormality. Ultrasound synovitis was reported as present or absent, and the definition of abnormality often included grade 1 greyscale synovitis only, which may be found in osteoarthritis and hypermobile joints as well as inflammatory arthritis. A recent cross sectional study, in 107 consecutive patients with musculoskeletal symptoms addressed these limitations by excluding patients with rheumatoid factor or cyclic citrulinated peptide and analysing according to OMERACT definitions [22,23]. Ultrasound changed clinical classification (synovitis/no synovitis) in 23% of patients. In total, 60% with inflammatory joint symptoms had no clinical synovitis, but of these 44% had any ultrasound synovitis [Grey Scale $\geq$ 2 or power Doppler (PD) $\geq$ 1], 28% had PD synovitis, 25% had severe PD synovitis, and 19% had tenosynovitis. Overall one in five symptomatic lupus patients has confirmed joint or tendon inflammation that is not detected clinically. Meanwhile, in 17% of patients with BILAG B or SLEDAI arthritis criterion, ultrasound was normal. In patients without clinical synovitis, inflammatory features on ultrasound appear in tendons more commonly than joints [24].

While prevalence of subclinical synovitis is agreed, it is not yet clear whether it can can truly account for symptoms and should be treated. Recent data indicate that patients with no joint swelling but subclinical synovitis on ultrasound have significantly worse tender joint counts than those with normal ultrasound [median (Interquartile range) 6(10) vs. 1(7), $P = 0.01$] [23]. Notably, although both patients and physicians rated musculoskeletal disease activity higher on a 0–100-mm visual analogue scale (VAS) when there was subclinical synovitis, the patients' median rating (55/100) was much higher than the physicians' (15/100). These results, therefore, suggest that physicians underrate disease activity in the absence of joint swelling. A pilot prospective study has suggested that ultrasound abnormality is more responsive than clinical outcome measures after glucocorticoid therapy [25].

Erosion

The presence of erosions using X-ray has been used as the key feature to differentiate Rhupus (deformities and erosions), Jaccoud's arthropathy (deformity but no erosion), and NDNE. The erosion data from imaging studies have revised this view. A large ultrasound study in an unselected lupus arthritis population, 87% of patients with Rhupus had erosions as expected. However, erosions were also found in 17 and 22% of the Jaccoud's and NDNE groups, respectively [24]. An MRI study found erosions in 45% of carpal bones, again present in all types of lupus arthritis. These rates of erosion also seem to exceed prevalence of Rhupus and Jaccoud's in their conventional definitions, so their clinical significance is less clear than in RA. Additionally, a detailed MRI study has suggested that their pathogenesis differs. Erosions in SLE patients are present in the absence of anticitrullinated peptide antibodies [26▪▪]. Anticitrullinated peptide antibodies in SLE patients were often reactive against the arginine-containing equivalent peptide, in contrast to RA [27]. In RA, synovitis then bone oedema are the precursors of bone erosion. However, in SLE many bones affected by erosion had either no synovitis, or synovitis at a level that would not lead to erosion in

an RA patient [26▪▪]. Although these RA and SLE populations had similar frequencies of erosions, bone oedema was significantly less frequent in SLE. Interestingly, interferon-β has been shown to inhibit osteoclastogenesis *in vitro*, therefore potentially retarding erosion (among other regulatory effects discussed below), although the relevance of this for human arthritis has not been proven [28,29].

IMMUNOPATHOGENESIS

At a molecular level, synovial gene expression studies in SLE patients demonstrate a distinct appearance from both osteoarthritis and RA. SLE synovium has marked upregulation of type I interferon-stimulated genes and downregulation of extra-cellular matrix homeostasis [30]. The stratification of SLE according to type I interferon status is increasingly important as this may predict response to a range of therapies [31]. The role of type I interferons in arthritis may be complex. Interferon (IFN)-α, primarily produced by circulating plasmacytoid dendritic cells and monocytes is generally associated with more severe disease in SLE [31]. However, although blood interferon activity is related to overall disease activity and individual organs such as mucocutaneous disease, it is not clearly related to arthritis [32]. Synoviocytes and fibroblasts produce interferon-β and this has been shown experimentally to have regulatory roles, with downregulation tumour necrotizing factor- α and upregulation of tumour growth factor- β, Interleukin (IL)-10, and IL-1ra [33–36]. Meanwhile, an interferon regulatory factor 5 risk haplotype for SLE is also associated with nonerosive rheumatoid factor-negative RA suggesting overlapping interferon-mediated pathogenesis [37], suggesting that a subset of RA patients have more SLE-like disease. Understanding the roles of type I interferons in SLE is of renewed interest as therapies that target this pathway are now in phase III trials and have demonstrated efficacy for arthritis-specific outcomes when targeting either (IFN)-α alone or the interferon receptor that is shared by IFN-α and IFN-β [38,39].

CLINICAL ASSESSMENT OF LUPUS ARTHRITIS

Recent clinical trials of biologics in SLE have led to a reappraisal of outcome measures and the definition of new composite endpoints. Lower frequency of clinical synovitis is a challenge in identification of patients amenable to immunosuppressive therapy, as well as in the assessment of response in clinical trials and routine practice [40]. In the various forms of the SLEDAI this is accounted for by the inclusion of erythema or warmth to define synovitis, as well as just joint swelling. In total, 4 points are scored for two or more joints with these signs (SLEDAI-2K) or more than two joints (SELENA–SLEDAI), and no points for lesser degrees of inflammation. However, these signs are more subjective than joint swelling and partial response cannot be captured. In clinical trials of belimumab an endpoint primarily based on the SLEDAI called the SLE responder index (SRI) was developed and has been used in trials of other agents [41]. The key criterion to meet this endpoint is a 4-point reduction in SLEDAI (qualified by no worsening in BILAG or Physician's Global Assessment). Hence, this criterion may be met by improvement in arthritis alone (even if disease in other organs remains active). However, meeting the criterion for arthritis response may not accurately capture all clinically meaningful change. Additionally, 4 points may be awarded for serological parameters, so patients who have no change in arthritis but with improvement in serological criteria may meet the SRI endpoint.

The BILAG-2004 index is semiquantitative for each organ system assessed. For the musculoskeletal domain, BILAG A (the highest score) requires observed active synovitis more than two joints with marked loss of functional range of movements. BILAG B is scored for tendonitis/tenosynovitis or active synovitis more than one joint (observed or through history) with some loss of functional range of movement (or improving BILAG A disease). BILAG C is scored for inflammatory pain (e.g. with morning stiffness) without synovitis (or improving BILAG B disease). Pain without inflammatory symptoms (e.g. pain that clinically appears to be because of osteoarthritis) is scored as BILAG D, as are patients with no current symptoms. Analogous to the SRI from the SLEDAI, the based combined lupus assessment (BICLA) is a clinical trials endpoint derived principally from the BILAG. BICLA requires reduction of BILAG A or B scores by at least one grade (qualified by no worsening in other BILAG domains, SLEDAI, physician global VAS or treatment failure).

Overall in scoring of arthritis, both these indices usually based on detection of swollen joints. The BILAG/BICLA allows response based on partial improvement in synovitis, unlike the SLEDAI. The BILAG C grade and SLEDAI 'tenderness, warmth, and erythema' criteria are similar in that they allow for scoring of subclinical synovitis. Only the SLEDAI/SRI allows this to qualify as treatment response.

THERAPIES FOR MUSCULOSKELETAL SYSTEMIC LUPUS ERYTHEMATOSUS

Although, arthritis is a common feature in clinical trials population, many trials have not analysed

response in individual organ domains. For conventional therapy of SLE, azathioprine, mycophenolate, or cyclophosphamide are frequently selected because of their clearly established efficacy, especially in renal disease. However, these agents tend to be less effective than methotrexate in RA and other inflammatory arthritis. For this reason, methotrexate is frequently suggested as a first-line immunosuppressive in lupus arthritis [40]. Evidence for the overall efficacy of methotrexate in nonrenal SLE is mixed. Steroid sparing, but not global (Systemic Lupus Activity measure and SLEDAI) disease activity reduction was found in one randomise control trial [42]. However, two smaller randomized trials did demonstrate efficacy in some arthritis-specific outcome measures [43,44].

Similarly, in trials of biologic therapies, some trials have reported organ-specific outcomes including arthritis. Of these, some studies show differences in efficacy between individual domains, especially comparing skin and arthritis. Arthritis is frequently one of the most responsive organ systems.

Belimumab is the only biologic licensed for lupus. Arthritis was the first and second most common manifestation at baseline in two phase III studies [45,46]. Individual BILAG domain responses were published as a post hoc analysis of the pooled population [47]. These results showed good efficacy in the musculoskeletal domain, with 60.7% of the patients with active disease at baseline (in the combined active arms) improving by at least one BILAG grade, compared to 50% of those on placebo. This was somewhat better than mucocutaneous disease (the next most common manifestation) which had improvement rates of 47.8 and 39.1% for the combined active and placebo groups, whereas no substantive difference between groups was observed for the less common domains of renal and haematological. Further, rates of worsening were reduced from 5.0 to 3.9/3.8% for the 1 and 10-mg/kg active groups in musculoskeletal disease. By comparison, rates of worsening in mucocutaneous domain were 4.5, 4.3, and 5.4% in the placebo, 1 and 10-mg/kg arms. Similarly, improvements were seen in associated quality of life domains of the Short Form Survey 36, such as physical function and bodily pain [48▪].

A flare study of abatacept in patients with arthritis, discoid lesions or pleuritis showed a reduction in BILAG-defined flare rate with abatacept for arthritis but not discoid lesions [49]. Our own group's open label data suggested a similar difference in efficacy using rituximab, with consistent efficacy in musculoskeletal disease but variable efficacy for mucocutaneous manifestations, with notable nonresponse in discoid lupus [50▪,51].

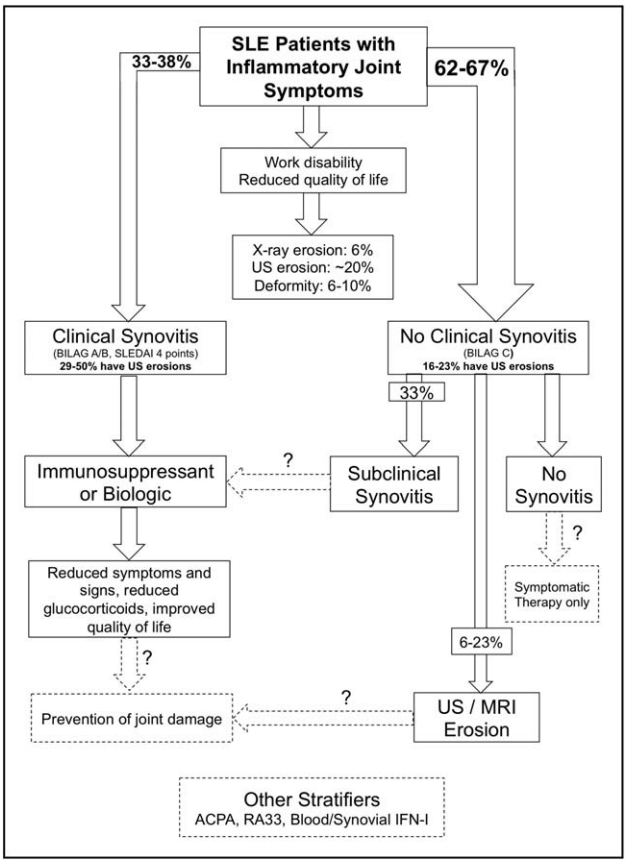

FIGURE 2. Current knowledge (solid arrows and boxes) and knowledge gaps (dotted arrows and boxes)in the treatment of inflammatory musculoskeletal SLE. See text for details. Key knowledge gaps are: (i) the value of immunosuppressive treatment for subclinical synovitis is not proven, nor the outcome of symptomatic treatment only in patients with normal imaging; (ii) erosions are more widespread than radiographic studies indicated but their long-term significance and any benefit of immunosuppression, are unknown; (iii) although SLE is heterogeneous for serology and interferon status, these stratifiers have not been investigated with respect to therapy. SLE, systemic lupus erythematosus.

Although, phase III trials for the B-cell-targeted biologic epratuzumab were negative, positive phase II data previously reported include efficacy in the musculoskeletal domain of BILAG [52]. Phase II data using the type I interferon-targeted biologics, sifalimumab, and anifrolumab included joint counts as secondary endpoints, demonstrating greater reductions in treatment groups compared to placebo [38].

Overall, arthritis often appears more responsive to immunosuppressive therapy than mucocutaneous disease (the next most frequent manifestation in all studies) using existing validated outcome measures. Further, arthritis was more uniformly

responsive to a wide range of conventional and targeted therapies.

SUMMARY OF CURRENT EVIDENCE FOR THERAPY

Evidence discussed in this review that might be used to inform treatment decisions is summarized in Fig. 2. Of patients with inflammatory joint symptoms, methotrexate and belimumab are effective for patients with synovitis in reducing symptoms and signs, glucocorticoid use, and improving quality of life. Similar benefits seem likely for other immunosuppressants and biologics based on various other types and strengths of evidence. That such therapy will reduce progression of erosions and deformity is not proven, but seems likely given the association of erosion with clinical synovitis in SLE, and parallel evidence in RA. This treatment pathway is, therefore, reasonably well proven in the context of SLE therapeutic evidence.

However, this pathway only applies to approximately one third of all SLE patients with inflammatory joint symptoms. The most appropriate therapeutic decisions are far less clear in the absence of synovitis. One third of these patients have subclinical synovitis on ultrasound. This subclinical synovitis group, therefore, represents the second largest subgroup of all SLE patients with joint symptoms, and a large proportion of SLE as a whole. This subgroup would not have met the inclusion criteria for clinical trials of immunosuppressive therapy or biologics and the long-term consequences or benefits of therapy are not well defined.

CONCLUSION

Modern therapies have dramatically reduced mortality in SLE but quality of life remains poor. Recent data suggest that changes to classification and assessment may allow improved outcomes using existing therapies.

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The authors confirm that this paper has not been published in its current form or a substantially similar form (in print or electronically, including on a web site), that it has not been accepted for publication elsewhere, and that it is not under consideration by another publication.
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Conflicts of interest

There are no conflicts of interest.

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Unraveling the pathogenesis of periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis through genetic, immunologic, and microbiologic discoveries: an update

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Purpose of review
Periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome is considered the most common periodic fever syndrome of childhood. Although it was first described three decades ago, the pathogenesis has been poorly understood. Recent studies on the heritability and immunology of the disorder have begun to shed light into the mechanisms of this autoinflammatory disorder. This review will focus on the pathogenesis of PFAPA, especially as it pertains to the genetic susceptibility, tonsillar immunology, and the role of the microbiome.

Recent findings
Recent literature provides insights into the heritability, potential genetic modifiers, and the immunologic and microbiological profile of the tonsils in this syndrome.

Summary
Evidence is mounting that PFAPA is inherited as a complex genetic disease. Furthermore, tonsillectomy is curative in the majority of patients, including those who do not meet the complete clinical criteria for PFAPA. The tonsils in PFAPA patients may exhibit unique immunologic and microbiological features. The goal of this review is to outline these new developments.

Keywords
autoinflammatory, periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis, tonsils

INTRODUCTION

Periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome was initially described in 1987 and is considered to be the most common periodic fever syndrome of childhood. The cause of this syndrome, however, remains a mystery. The genetic heritability of the disorder has been controversial, but recent studies on heritability and the search for candidate genes have begun to provide some clues into the pathogenesis of this disorder. In addition, recent studies support the curative role of tonsillectomy and show the immunologic and microbiome profiles in the tonsils of patients with PFAPA. The diagnosis of PFAPA is based on the criteria proposed by Thomas *et al.* [1] (Table 1).

HERITABILITY OF PERIODIC FEVER, APHTHOUS STOMATITIS, PHARYNGITIS, AND CERVICAL ADENITIS SYNDROME

Since the initial report of PFAPA, several familial cases have been described in the literature particularly among siblings and parent–child pairs [2–5]. Prior to these case series, PFAPA was thought to be a sporadic disease [6]. Moreover, the syndrome does not appear to cluster in particular ethnic groups with cases reported from the United States, Japan, Europe, South America, and the Middle East, suggesting the lack of a common genetic susceptibility factor [1,5,7–9]. However, recent descriptions of cohorts report that 10–78% of patients have a family member with 'recurrent fever' suggesting that

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KEY POINTS

- Heritability studies of familial clustering suggest that PFAPA may be inherited in an autosomal dominant pattern with many family members having reduced penetrance phenotypes.
- Inflammasome/IL-1β pathway genes and genes involved in other periodic fever syndromes may modulate disease manifestations in some populations of patients with PFAPA.
- Tonsils from patients with PFAPA had a lower percentage of B lymphocytes and higher percentage of some T lymphocyte subsets from patients with sleep apnea.
- Tonsils from patients with PFAPA may have a unique microbiome composition.

familial clustering may be more common than originally thought [10–13].

Recently, two groups have performed more comprehensive assessments of inheritance [14▪▪,15▪▪]. Manthiram *et al.* [15▪▪] systematically evaluated the family history in 80 patients with PFAPA and found that 18 (23%) had at least one family member with symptoms suggestive of PFAPA, with most of the family pedigrees suggesting an autosomal dominant inheritance pattern. However, affected individuals within families did not show strong concordance in terms of episode characteristics such as age of onset or length of intervals between episodes; implying that environmental factors might influence the phenotypic manifestations of the disease. Familial clustering alone does not prove that a disease is inherited, as it can also indicate common environmental triggers shared among family members. However, in this cohort, living in the same household did not significantly increase the likelihood of a first-degree or second-degree family member having PFAPA or PFAPA-like features.

In comparison with healthy controls, Manthiram *et al.'s* PFAPA cohort was significantly more likely to have first-degree family members with recurrent pharyngitis, recurrent aphthous stomatitis, or both. In addition, siblings of children with PFAPA were significantly more likely to have undergone tonsillectomy. These data informed the hypothesis that family members with recurrent pharyngitis or recurrent aphthous ulcers (who do not meet the strict diagnostic criteria for PFAPA) may represent reduced penetrance phenotypes of PFAPA syndrome. Di Gioia *et al.'s* analysis of 14 families of PFAPA patients showed an autosomal dominant inheritance pattern with an estimated penetrance factor of 50% if Mendelian inheritance was assumed.

Both of these studies clearly show familial clustering with an autosomal dominant inheritance pattern, suggesting that susceptibility to PFAPA may be inherited. However, the presence of many family members with incomplete penetrance phenotypes and poor concordance of episode characteristics among affected family members do not diminish the role environmental influences may play in disease manifestations. These studies also highlight the importance of careful questioning of families about relatives with not only recurrent fever, but also with recurrent aphthous stomatitis and/or recurrent pharyngitis/tonsillitis, since these findings were common in family members of patients with PFAPA.

GENOMIC ANALYSIS OF PERIODIC FEVER, APHTHOUS STOMATITIS, PHARYNGITIS, AND CERVICAL ADENITIS

Di Gioia *et al.* [14▪▪] recently published results of their exhaustive genetic screening techniques to identify causative mutations in patients with PFAPA. Genome-wide linkage analysis in seven families revealed a single peak on chromosome 8 (8q21.1–8q24.4) with a logarithm of the odds score of 2.9. However, complete sequencing of all exons in that region in three individuals with PFAPA did not reveal variants in the coding section of a common gene. Whole exome sequencing of 11 individuals with PFAPA and one unaffected individual, with filtering for rare variants and a minor allele frequency of less

Table 1. Original diagnostic criteria for periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis

I	Regularly recurring fevers with an early age of onset (<5 years of age)
II	Constitutional symptoms in the absence of upper respiratory infection with at least one of the following clinical signs: aphthae cervical lymphadenitis pharyngitis
III	Exclusion of cyclic neutropenia
IV	Completely asymptomatic interval between episodes
V	Normal growth and development

than 2%, revealed 14 candidate genes with variants detected in more than 90% of individuals sequenced. However, none of these genes were predicted to be causal for PFAPA since variants were also sequenced in unaffected family members, considered sequencing artifacts, or present in highly polymorphic genes. With their genomic analysis, the authors concluded that PFAPA is likely a complex disease of oligogenic or polygenic inheritance or a group of Mendelian diseases with a common phenotype. Bens *et al.* [16] reported one patient with PFAPA and dysmorphic features with a de-novo chromosomal translocation causing a microdeletion in chromosome 17 containing a gene called *SPAG7*, which is present in lymphoid tissue. However, in the larger cohort of patients studied by Di Gioia *et al.*, mutants in the *SPAG7* gene were not found.

Considering that PFAPA has clinical features similar to the known monogenic periodic fever syndromes and patients appear to have activation of IL-1β and inflammasome-related pathways during flares; genes related to these pathways have been selectively studied as potential modifier genes in patients with PFAPA [17,18]. Patients with pathogenic mutations in monogenic periodic fever syndrome genes but clinical features of PFAPA have been described. Those with clinical symptoms of PFAPA with pathogenic variants seen in known hereditary periodic fever syndromes more frequently had symptoms outside of the oropharynx during flares such as abdominal pain, vomiting, diarrhea, rash, and arthralgia underscoring the importance of obtaining genetic testing in those with atypical features [19]. In this same study, patients with variants of unclear significance were not significantly different from those without variants. In an Israeli study of 124 patients with a clinical diagnosis of PFAPA and lacking features of familial Mediterranean fever, patients who were heterozygous for common *MEFV* variants (M694V, V726A, and E148Q) had episodes that were shorter in duration, had less regularity in timing, and were less likely to have associated aphthous stomatitis, suggesting that variants in *MEFV* may influence the clinical phenotype of PFAPA [20]. Another group in Japan also reported that PFAPA patients with *MEFV* variants had shorter episode durations [7]. However, these findings were not replicated in another cohort of 64 patients in Turkey [21]. Therefore, the role of *MEFV* as a disease-modifying gene in PFAPA remains unclear.

Screening for mutations in *MEFV*, *TNFRSF1A*, *MVK*, *NLRP3*, *AIM2*, and *NOD2/CARD15* in PFAPA cohorts (ranging in size from eight to 124 patients) in Israel, Turkey, Italy, Slovenia, and Switzerland have not consistently shown a higher prevalence of variants in these genes in PFAPA patients compared with controls [7,13,14▪▪,17,20–24▪] (Table 2). However, these studies varied in how extensively these genes were sequenced for polymorphisms and in the allele frequency among healthy people of different ethnic backgrounds. A high prevalence of *NLRP3* variants were found in PFAPA patients in Switzerland (15 out of 57 patients), and *MEFV* variants in a Turkish population (42 out of 64 patients); however, this was not seen in other populations, nor compared with the prevalence of these variants in controls in these studies [17,21]. Several rare variants in inflammasome and monogenic periodic fever syndrome-related genes were identified by Di Gioia *et al.* in 11 individuals who underwent whole exome sequencing, but the functional significance of these variants is unknown.

Recently, Cheung *et al.* [24▪] reported that nearly 14% of patients in their cohort of 82 unrelated PFAPA patients carried a frameshift mutation in *CARD8* (CARD-FS) in comparison with 3.2% of healthy controls. The protein encoded by *CARD8* interacts with the NLRP3 inflammasome and inhibits its ability to activate caspase-1. Caspase-1 is necessary to cleave pro-IL-1β to its active form, IL-1β. The authors found that HEK298T cells transfected with the CARD-FS mutant and inflammasome components lacked the NLRP3 and CARD8 interaction which would presumably lead to NLRP3 activation. Patients with *CARD8* mutations were more likely to have aphthous ulcers and symptoms between flares. However, another common polymorphism in the *CARD8* gene (C10X) was not found to be more common in PFAPA patients in comparison with healthy controls.

These recent advancements reveal that although PFAPA clusters in families and may be inherited, the syndrome is unlikely to be a monogenic disease in most patients. However, inflammasome/IL-1β pathway genes and genes involved in other periodic fever syndromes may modulate disease manifestations in some populations.

EFFICACY OF TONSILLECTOMY

Shortly after PFAPA was initially described, reports of the success of tonsillectomy in achieving full resolution of the syndrome were published. Two randomized trials comparing tonsillectomy with no surgical therapy demonstrated that patients in the tonsillectomy arm were significantly more likely to have complete resolution of episodes [25–27]. In a subsequent larger cohort of 102 PFAPA patients who underwent tonsillectomy, 97% had complete resolution of episodes after an average of 4 years of

Table 2. Genomic analyses in periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis

Reference	Country	Number of patients	Genes or variants analyzed	Gene frequency	Clinical findings
Maschio *et al.* [23]	Italy	40	*CARD15/NOD2*: R702Q, G908R, 1007fsinC	*CARD15*: no patients had variants	
Dagan *et al.* [22]	Israel	57	*MEFV*: M694V, V726A, E148Q, M694I, M680I	16/57 with variant in *MEFV*	No difference in clinical features of those with and without variants
			TNFRSF1A: P46L, R92Q	3/57 with variant in *CARD15/NOD2*	
			CARD15/NOD2: LfinsC1007P, R702W, G908R	1/57 had variant in *TNFRSF1A*	
			NLRP3: L353P	None had variants in *NLRP3*	
				These frequencies were same as those expected in healthy people in the population	
Berkun *et al.* [20]	Israel	124	*MEFV*: M694V, V726A, E148Q in all and M680I and M694I in non-Jewish population	65/124 had one *MEFV* variant (most common variant M694V), which is comparable with the gene frequency in the population	Those with *MEFV* mutations had shorter episodes, more irregularity in timing, and less aphthous ulcers
Kolly *et al.* [17]	Switzerland	57	*MEFV*: exons 2 and 10	4/57 had variant in *MEFV*	Those with any variant were more likely to have family history of recurrent fever
			TNFRSF1A: exons 2, 3, 4, 6	12/57 had variant in *NLRP3*	
			MVK: exons 9 and 11	1/57 had variant in *TNFRSF1A*	
			NLRP3: exon 3	1/57 had variant in *MVK*	
				Variants in *NLRP3* were more frequent than expected in healthy population	
Taniuchi *et al.* [7]	Japan	20	*MEFV*: All exons	13/20 had one *MEFV* variant (most common was E148Q-L110P)	Those with *MEFV* mutations had shorter episode duration
			MVK: All exons	None had variants in *MVK* or *TNFRSF1A*	
			TNFRSF1A: All exons	In comparison with healthy controls, only the frequency of E148Q-L110P variant was significantly higher in PFAPA (35 vs. 13%)	
Perko *et al.* [13]	Slovenia	81	*MEFV*: all exons	5/62 had variant in *MEFV*	
			NLRP3: entire gene	13/62 had variant of clinical significance in *NLRP3*	
			MVK: all exons	No patients had variant in *MVK*	
			AIM2: entire gene and promoter	No patients had variants of clinical significance in *AIM2*	
Celiksoy *et al.* [21]	Turkey	64	*MEFV*: exons 2, 3, 5, 10	42/64 had variants in *MEFV*, which is higher than expected in the Turkish population	No difference in the clinical features of those with and without variants

Table 2 (*Continued*)

Reference	Country	Number of patients	Genes or variants analyzed	Gene frequency	Clinical findings
Di Gioia 2015 [14▪▪]	Switzerland	8 families (11 individuals)	WES in families and some individuals, looked specifically genes connected to autoinflammatory diseases	2/8 had variant in *MEFV* 1/8 had variant in *NLRP3* 2/8 had variant in *TNFRSF1A* 1/8 had variant in *NLRP12*	
Cheung *et al.* [24▪]	Switzerland	82	32 genes involved in known autoinflammatory syndromes or associated with the inflammasome including *CARD8*	Only reported frequency of variants in *CARD8* variants: 12/82 had variants in *CARD8*, which is more than controls	Those with *CARD8* frameshift mutation were more likely to have symptoms outside of flares and aphthous ulcers

WES, Whole exome sequencing.

follow-up [28]. This work has been extended in a recent article that characterizes the role of tonsillectomy in the management of patients who do not fully meet the diagnostic criteria for PFAPA [29]. Lantto *et al.* followed 108 patients who had undergone tonsillectomy for regularly recurring fever at their medical center in Finland from 1990 to 2007.

Fifty-eight of these 108 children met the strict criteria for PFAPA as outlined in Table 1, whereas the remaining 50 had only regularly recurrent fever (lacked pharyngitis, adenitis, or aphthous stomatitis during flares) and were defined as incomplete. In the 58 with strictly defined PFAPA, 97% (56/58) had complete resolution of fever episodes after tonsillectomy, and in the 50 patients in the incomplete group, all had complete resolution of fever episodes after tonsillectomy. The investigators also noted that children with a late onset of symptoms (>5 years of age) also had an excellent response to tonsillectomy. The authors concluded that tonsillectomy was an effective treatment for patients with both strictly defined and incomplete PFAPA.

TONSIL IMMUNOLOGY AND MICROBIOME

The curative role of tonsillectomy in PFAPA has focused attention on understanding the pathophysiology in patients' tonsils that may be triggering episodes. Comparisons of cytokine transcript expression in PFAPA tonsils and obstructive sleep apnea/hypertrophic tonsils show that PFAPA tonsils express less IL-4 [30]. To further dissect the role of the tonsils, Dytrych *et al.* [31▪▪] performed a more comprehensive assessment of lymphocyte subsets by flow cytometry in both the blood and removed tonsils of 10 patients with PFAPA and compared them with samples obtained from patients with obstructive sleep apnea. The PFAPA tonsils had a lower percentage of B lymphocytes, higher percentage of CD8+ T lymphocytes, and higher percentage of naïve CD4+ and CD8+ T lymphocytes than tonsils from patients with sleep apnea. In addition, tonsils from patients with PFAPA had fewer CD4+ T-lymphocytes with high expression of the inhibitory molecule PD-1. T-cell chemokines levels were also elevated in PFAPA tonsils. Immunoglobulin and T-cell receptors did not show clonal or oligoclonal expansion. Significantly, prior studies show that in peripheral blood, patients with PFAPA have fewer CD4+ and CD8+ T lymphocytes and elevated levels of T-cell chemoattractants during flares in comparison with asymptomatic intervals [18].

These results suggest that naïve, polyclonal T lymphocytes accumulate in the tonsils from the peripheral blood as part of the pathogenesis of PFAPA; this accumulation may subsequently affect B-cell

development in the tonsils as well. Many questions remain unanswered, including what triggers the T-cell influx into tonsils, what effect this influx has on B lymphocytes, how it triggers inflammation in the tonsil, what role innate immune pathways and cells play in the pathogenesis, what feedback loops lead to regular cyclic episodes of tonsillar inflammation, and why these inflammatory cycles are unique to the oropharyngeal lymphoid tissue. The efficacy of tonsillectomy among patients who do not strictly meet the criteria for PFAPA and the high frequency of presence of individuals with reduced penetrance phenotypes suggest that a broader array of phenotypes may fall under the umbrella of PFAPA. Other recent studies also recognize the heterogeneity of the syndrome [8,32].

The microbiome of the tonsils has been explored as an inflammatory stimulus or disease modulator. Dytrych *et al.* [31▪▪] assessed the viral load of Epstein–Barr virus, cytomegalovirus, human herpervirus-6, and adenovirus in the tonsils of patients with PFAPA and controls by quantitative PCR. They detected at least one of these viruses in seven of 10 PFAPA tonsils but also in seven of nine controls. Lantto *et al.* [33] performed bacterial, viral, mycobacterial, and fungal cultures as well as PCR for herpes viruses. In addition, they visualized biofilms in tonsils of 31 PFAPA patients and 24 patients with obstructive sleep apnea. They found that the tonsils from PFAPA patients were more likely to contain *Candida albicans* and develop biofilms and less likely to contain *Staphylococcus aureus* and varicella-zoster virus. This same group recently published a follow-up study using next-generation sequencing of 16S ribosomal RNA to more thoroughly profile the bacterial microbiota from tonsils removed from the same 30 PFAPA patients and 24 controls [34▪▪]. These studies found no phylum, genera, or species that were present in all PFAPA tonsils and absent in all the obstructive sleep apnea patients, and no differences in microbial populations by principal component analysis. However, the proportions of samples that tested positive for and the relative abundance of particular phyla, genera, and species differed significantly between the PFAPA cases and sleep apnea controls. At the phylum level, PFAPA tonsils were more likely to contain cyanobacteria and *Synergistetes* than controls. At the genera level, the mean relative abundance of streptococci was lower and that of *Prevotella* was higher in the cases than in controls. No differences in the frequency of nasopharyngeal pathogens like *Haemophilus* and *Mycoplasma* were found between cases and controls; moreover, with sequencing, differences in the presence of *S. aureus* that were detected by culture were not found. The lack of identification of a common microbe in the tonsils of PFAPA suggests that PFAPA is not an infectious disease although thorough assessments of the virome and fungi have not yet been conducted. Nevertheless, it remains unclear whether differences in the tonsil microbiome play a causal role in stimulating the disease, or whether the differences are a result of repeated episodes of inflammation.

In addition, all tonsil studies to date are limited by the removal of tonsils only during asymptomatic periods and comparison with tonsils from patients with obstructive sleep apnea and/or hypertrophic tonsils, which are inflammatory diseases as well. Longitudinal assessments of the tonsil microbiome over time may provide additional information. Understanding tonsillar immunology and microenvironment is a valuable pathway to unraveling the pathogenesis of PFAPA.

CONCLUSION

PFAPA has many unique aspects, including the regularity of episode timing, the central role of the palatine tonsils, and the familial clustering of the disorder. Recent developments in unraveling the pathogenesis of PFAPA have begun to elucidate the complex genetic and immunologic mechanisms underlying the syndrome. Genomic analyses in larger populations may help identify common variants with low penetrance, whereas large families with severe disease may help identify rare, causal variants that illuminate components of the mechanism. Future studies investigating the functional implications of these variants should be studied in the tonsils to help detangle the intricate web of genetic complexity with a common phenotype.

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Conflicts of interest

There are no conflicts of interest.

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- ▪ of special interest
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Precision medicine in pediatric rheumatology

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Purpose of review
Precision medicine is the tailoring of medical care to subcategories of disease. In pediatric rheumatology, these subcategories must first be defined by their specific molecular immunological profiles, and then the effects of growth and puberty, developmental immunological changes, and differences in treatment options and adherence considered when designing therapeutic strategies. In the present review, we summarize the unmet needs in pediatric rheumatology before such precision medical care can be effectively delivered to affected patients.

Recent findings
The current clinical classification of pediatric rheumatic diseases does not provide all the information necessary for prognostication and accurate therapeutic selection. Many studies have highlighted the molecular differences between disease subcategories and the dissimilarities in the molecular manifestations of the same disease between patients. Harnessing such discoveries by collaborating with various research networks and laboratories is required to interrogate the multifactorial nature of rheumatic diseases in a holistic manner.

Summary
Integration of big data sets generated from well defined pediatric cohorts with rheumatic diseases using different high-dimensional technological platforms will help to elucidate the underlying disease mechanisms. Distilling these data will be necessary for accurate disease stratification and will have a positive impact on prognosis and treatment choice.

Keywords
disease stratification, omics, pediatrics, precision medicine, rheumatology

INTRODUCTION

The therapeutic strategies applied to pediatric rheumatologic disorders have undergone rapid development over the past decade because of the implementation of a clearly defined and early, treat-to-target approach and the increasing availability of effective biologics. Such biologics have been based on strategies like tumor necrosis factor-α (TNF-α) blockade, interleukin-1 and interleukin-6 inhibition, and B-cell depletion, and have permitted patients who would otherwise be resistant to traditional disease-modifying antirheumatic drugs or immuno-suppressants to achieve inactive disease or remission status. Despite these advances, hurdles still exist in the application of precision medicine to children with rheumatologic disorders namely because of suboptimal diagnosis, subclassification of disease, disease monitoring, and treatment stratification. To improve clinical outcomes in this population, the effects of growth and puberty, developmental changes in immune cell subsets, and the differences in treatment options and adherence between pediatric and adult populations should be considered [1]. Furthermore, exposure to unnecessary or ineffective medications must be reduced and an optimal therapeutic response achieved with minimal adverse effects. By defining each subcategory of disease by its unique immunologic profile and incorporating individual genomic, epigenetic and environmental influences, we hope to overcome the challenges in this field and ensure that medical care is precisely tailored to the individual patient.

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KEY POINTS

- Precision pediatric rheumatology is the providence of tailored medical care to subcategories of patients based on a molecular taxonomy disease classification.
- Precision pediatric rheumatology must be independently studied, as genetic factors, growth and puberty, developmental changes on the immune cell subsets, and differences in treatment adherence can affect the clinical outcomes.
- Procedural standardization of patient identification, data and sample collection, and integration with data obtained from holistic, multiplatform interrogation of biological samples will provide the best avenue to elucidate the multifactorial mechanisms underlying these diseases.

CURRENT SHORTFALLS IN PRECISION PEDIATRIC RHEUMATOLOGY

As discussed, there remain numerous obstacles to implementing precision medicine in rheumatologic disorders in the pediatric population. Here, we appraise the limitations to the current disease taxonomy system, and consider the development of a new, comprehensive disease taxonomy that incorporates genetic and biomarker data. In addition, we discuss the necessary improvements in assessing disease activity, and the generation of new tools to predict treatment responses.

Disease taxonomy

We first propose that a new, multidimensional taxonomy of disease classification is required to refine or challenge the traditional classification of pediatric rheumatologic diseases. The current disease classifications are based mainly on anatomical, histological, and clinical features but they do not take into consideration the underlying pathophysiological mechanisms that can influence therapeutic strategies [2,3]. A new taxonomy system would integrate the most recent, clinically relevant scientific discoveries with unique or notable biological significance, which could then be translated into clinical practice. For example, the influence of certain genetic polymorphisms in conferring susceptibility to various rheumatic diseases is stronger in pediatric than adult populations. Although genetic associations with various autoimmune diseases have been long established (such as major histocompatibility complex), genotyping at this level currently has a limited role in diagnosis or subcategorization of disease.

The integration of genetic data with disease taxonomy systems may increase in the near future, particularly for juvenile idiopathic arthritis (JIA). A recent genome-wide association study (GWAS) demonstrated the lack of shared genetic risk factors between systemic JIA and other subtypes of JIA, suggesting that systemic JIA is a distinctive disease that warrants a different classification framework [4▪▪]. Incorporating such genetic data into disease classification systems will increase the accuracy of diagnoses and will circumvent the limitations of current clinical classifications, such as for JIA whereby the disease is currently subcategorized based on the number of joints involved, serology, and extra-articular manifestations [5].

Incorporation of genetic data may also be of value in predicting disease prognosis. Studies have demonstrated that childhood onset of systemic lupus erythematosus (SLE) is associated with a higher number of known SLE-susceptibility risk alleles compared to the adult counterpart [6]. This higher number of alleles may partially explain why children with SLE experience a more severe and aggressive course compared to adults and frequently present with major organ involvement [7].

Biomarker identification

We secondly consider the urgent need for better tools to evaluate and predict treatment responses, namely those that consider the differences in therapeutic responses to the same drug between patients with the same clinical diagnosis or disease subtype. In day-to-day practice and in clinical trials, validated global activity scores in pediatric rheumatic diseases are commonly used as surrogates to measure disease activity and response to therapy. Because of the systemic nature of many rheumatic diseases, however, the same score with the same global disease activity measure (such as SLE disease activity index), either from the same patient at a different time point or between different patients, can reflect an entirely different clinical picture. Global disease activity scores cannot describe the diverse underlying immuno-pathogenic mechanisms that occur in different organs or in different patients. Furthermore, routinely available laboratory tests, such as estimation of erythrocyte sedimentation rate or C-reactive protein levels, reflect global inflammation and are not disease specific. Even disease-specific disease activity markers (such as anti-dsDNA, complement levels in SLE) are not optimal as they lack specificity at the organ level.

The search for biomarkers has attracted notable interest in the research community, and although some soluble biomarkers have been identified in pediatric rheumatology – including heme-oxygenase 1, interleukin-6, interleukin-12, interleukin-18,

osteoprotegerin, S100 calcium-binding protein A12 (S100A12) and S100A8/A9 in systemic JIA and urinary biomarkers in childhood lupus nephritis – they have not yet been incorporated into routine day-to-day disease management [8,9]. We propose that a shift in focus from soluble inflammatory mediators to the specific pathologic cellular subsets that secrete these mediators in the various microcompartments (synovium, synovial fluid, lymph node, and other specific organ sites) will likely yield more biologically important and clinically relevant information.

By understanding the mechanisms that explain the differences in therapeutic response and identifying the distinct differences that exist within a common biological phenotype, we may be able to compartmentalize patients into distinct subgroups based on the same diagnosis, which will facilitate therapeutic selection. For example, methotrexate is an effective therapeutic for JIA but only 30–50% patients have a sufficient response to this drug and many children develop adverse effects [10,11]. This phenomenon might be partially explained by genetic factors that influence methotrexate metabolism, and candidate-gene and genome-wide pharmacogenomic studies in JIA have identified single nucleotide polymorphisms (SNPs) and gene regions that are potentially associated with response or toxicity to methotrexate [12]. Although many of these SNPs have not been replicated in other cohorts or shown consistency, it may explain in-part, the heterogeneous response exhibited by patients with JIA treated with methotrexate.

The ability to characterize the underlying pathogenic mechanisms responsible for disease manifestation will also enable us to define a clear treatment endpoint and identify an objective outcome measure that represents a 'normal' immunological status and indicates successful disease therapy. Such identifiers will also distinguish true remission from suppressed disease activity, and help determine when halting treatment is considered clinically appropriate. Defining these pathogenic mechanisms at the molecular level may even advance the development of novel drugs, or the repurposing of currently available biologics.

RECENT ADVANCES IN PEDIATRIC RHEUMATOLOGY

Assessment of disease activity and prediction of treatment response in heterogeneous pediatric diseases, such as JIA and SLE, requires the imputation of information obtained from various 'omic' datasets. Thus far, molecular parameters such as genetic profiles, gene and protein expression, and cellular immune-phenotyping have not been readily translated into clinical practice. A few studies, however, have indicated the importance of including such molecular and genetic data in clinical management, as will be discussed below.

Transcriptome analyses

A recent transcriptome analysis demonstrated that downregulation of specific innate immune-response genes in patients with systemic JIA, including those associated with interleukin-1 and interleukin-6 signaling, occurred following canakinumab treatment. Of notable clinical importance was that the strongest clinical response to canakinumab was associated with higher baseline expression of systemic JIA-induced specific genes and marked transcriptional reduction in multiple genes notably interleukin-1 related (interleukin-1β, interleukin-1 receptors, interleukin-1 receptor accessory proteins) and interleukin-6 at day 3. This effect was evident even prior to the assessment of the primary clinical outcome on day 15 [13▪].

Innovative immunologic approaches

Using innovative immunologic approaches (based on T-cell receptor sequencing), our center recently identified a small but unique subset of circulating $CD4^+$ T cells that exhibited a phenotypic signature representative of lymphocytes that infiltrated the inflamed synovium in patients with JIA and rheumatoid arthritis. These circulating pathogenic-like lymphocytes were pro-inflammatory, enriched in synovial clonotypes, and most notably, expanded in patients with JIA who did not respond to conventional treatment [14]. These data identified a circulating population of cells associated with active disease and could be potentially targeted for diagnostic and therapeutic purposes with further studies.

Gene expression analysis

Data are emerging to suggest that there are mechanistic differences that underlie the systemic manifestation of SLE and its renal involvement (lupus nephritis). The kidney is an important organ-specific manifestation of SLE and a major determinant of long-term outcome [15]. Although many potential noninvasive urinary biomarkers have been identified, including leukocytes, chemotactic proteins involved in the recruitment of inflammatory cells [monocyte chemoattractant protein 1, C-X-C motif ligand (CXCL) 10, and CXCL16], and products of immune cells (TNF-like weak inducer of apoptosis), none of these can be used to definitively

distinguish between the different histopathological classes of lupus nephritis and provide the necessary mechanistic insight that will influence therapeutic choice [8,16]. Of promise, however, was the identification of increased expression of platelet-derived growth factor (PDGF) and human epidermal growth factor receptor 2 (HER2) in mesangial cell proliferation – an important feature of lupus nephritis [17,18]. HER-2 is of great interest in the rheumatologic setting because of: its specific association with pediatric lupus nephritis, compared to PDGF that is also associated with other causes of nephritis; interferon-α-mediated upregulation; its defined mechanism of enhancing mesangial cell proliferation secondary to its repressive effects on microRNA species (miRNAs miR-26a and miR-30b) that regulate the cell cycle; and the availability of an anti-HER2 monoclonal antibody (trastuzumab) currently used in an oncologic setting, which could potentially be repurposed for lupus nephritis [18].

GENERATION OF LARGE DATASETS USING MULTIDIMENSIONAL MOLECULAR PLATFORMS

The highlighted limitations indicate the acute need to identify the unique molecular differences between individuals with the 'same disease' or 'disease subtype' in order to permit patient stratification. In this way, diagnosis, treatment strategy, and timing of drug withdrawal may be more accurately considered. To achieve the goal of precision rheumatological care, we must move from conventional and oligo-dimensional analyte and cell-based research to the application of multidimensional and high-throughput platforms that are currently available in the different 'omic' workflows. For immunomics specifically, this will involve high-throughput platforms such as cytometry by time-of-flight (CyTOF) to identify target cell populations of clinical and mechanistic importance so that fluorescence-based flow sorting of these cells can be done for deep RNA sequencing to identify the deranged signaling pathway for potential therapeutic target in addition to the use of these data for patient stratification.

Cytometry by time-of-flight datasets in pediatric rheumatology

CyTOF is a revolutionary technology that permits deep interrogation of immune subsets using cell lineage and functional markers. By this method more than 30 biomarkers can be simultaneously detected at the single-cell level. Our laboratory designed a panel of 37 markers to study the T-cell compartment in JIA by CyTOF, using samples collected from subjects recruited for a clinical trial studying the effects of anti-TNFα biologics. Our analysis identified that the patients who experienced a disease flare within 6 months of drug discontinuation exhibited an enrichment in pro-inflammatory, antigen-stimulated T cells that were preexisting prior to the withdrawal of therapy [19]. The phenotype composed of $CD3^+CD4^+$ memory ($CD45RA^-$) antigen experienced ($CD40L^+$, $CD69^+$) T cells that expressed costimulatory ($CD28^+$, $ICOS^+$) and immune checkpoint ($CTLA4^+$) markers and were capable of secreting high levels of TNF-α. Intriguingly, $CD3^+CD4^+CD25^{hi}$-$Foxp3^{hi}CD45RA^-CTLA4^{hi}CD28^+ICOS^+$ cells were found to be significantly upregulated within the T regulatory cell compartment of patients experiencing a disease flare. This effect may be indicative of an inadequate attempt to downregulate the inflammatory response [19]. These findings have identified some of the disease-associated cellular subsets that may be potentially used for clinical prognostication and further interrogation of the function of these cells may elucidate the responsible cellular pathways for therapeutic targeting. Such a holistic approach has the potential to elucidate the pathologic mechanisms underlying JIA in an efficient manner for the subsequent development of appropriate therapeutics.

Integration of omic-derived datasets

Although the relative rarity of many rheumatological diseases has presented some hurdles to the progression of research, advances in available technological platforms have created opportunities for collaboration between different fields of study and has encouraged the development of analytical pipelines that facilitate the interpretation of big datasets. The data derived from individual 'omics' platforms has been instrumental in enhancing our understanding of rheumatological diseases, but it is the integration of these datasets in the study of well characterized patient groups, obtained from different geographical regions and networks, that will provide a collective mechanistic insight into these diseases (Fig. 1).

A distinct example of such integration was the mapping of predicted disease susceptibility SNPs from GWAS of different rheumatological (including pediatric) diseases to epigenomic markers of an active *cis*-regulatory element in an immune cell and stimulation-specific manner; this provided an insight into the effects of genetic variations on gene expression [20]. Majority of the SNPs (60%) are mapped to immune cell enhancers suggestive of a gene regulatory role and this provides an initial step

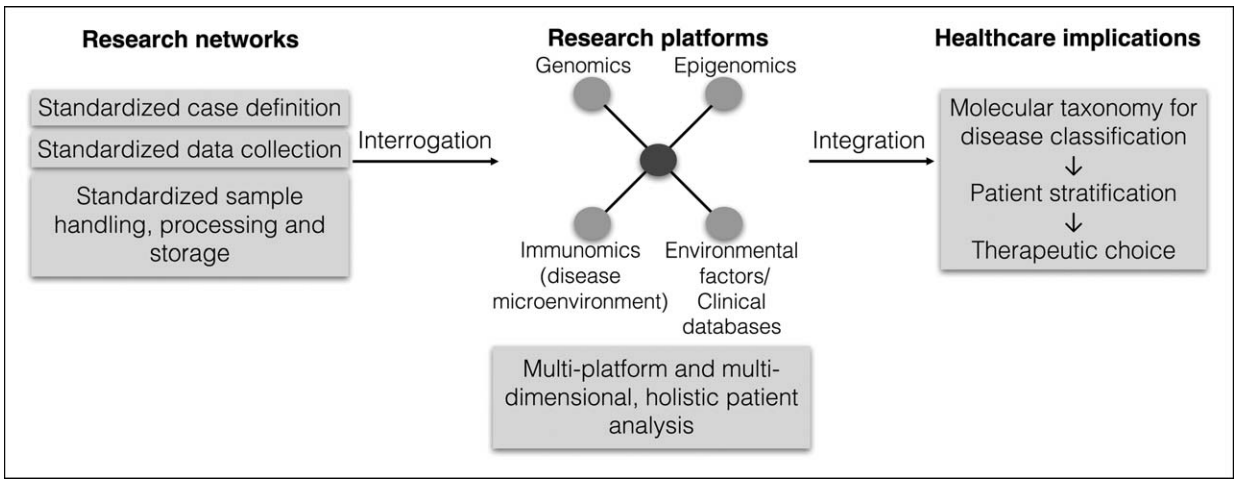

FIGURE 1. Integrative holistic translational research approach. Collaboration between different research groups that provide clinically well defined patient cohorts coupled with good quality bio-specimens will allow deep interrogation with different research platforms. The intersection and integration of these big datasets provides a holistic view of the underlying mechanistic differences and evidence to influence clinical decisions.

in the effort to establish the underlying mechanism and causality of these genetic links to immune regulation. Further research efforts of this nature are now required in order to dissect the complexity of autoimmune diseases for developing novel prognostic, diagnostic, and therapeutic approaches.

CONCLUSION

We strongly propose that the Holy Grail in precision rheumatology lies at the interface of large data sets obtained from well characterized pediatric rheumatologic cohorts, and that this integration will provide important mechanistic insights. This integration is necessary because of the multifactorial nature of rheumatic diseases where genetic, epigenomic, and environmental influences all contribute to immune derangements. Resources must become available to standardize and harmonize research procedures, including sample collection and handling, data analysis, storage, and sharing such that high-quality datasets can be generated for meaningful comparison. Several best practices and guidelines are available for reference, including the International Society for Biological and Environmental Repositories (ISBER), Biobank Standardisation and Harmonization for Research Excellence in the European Union (BIOSHARE-EU), UK Biobank, and Understanding Childhood Arthritis Network (UCAN) [21▪]. Furthermore, electronic health records contain dynamic longitudinal information on clinical parameters, routine laboratory investigations, disease activity scores, family and environmental history, prescribed medications, and comorbidities. The innovative and precise integration of the information contained within these records with laboratory-derived omic datasets is urgently needed to catalyze precision medicine in pediatrics. By aggregating individual datasets into big data complex algorithms, more robust information can be provided to clinicians to facilitate clinical decision-making and ultimately lead to improved patient care and outcomes. The smooth integration of healthcare records and large datasets from the laboratories will require a concerted effort and collaboration between various stakeholders to navigate and overcome the challenges. It is hoped that the integration of these large datasets will provide a complete mechanistic picture of these diseases, which will be helpful for patient stratification.

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Conflicts of interest

There are no conflicts of interest.

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This perspective article highlights the factors for standardization and best practices to adopt to ensure reproducibility of results in collaborative research.

No shortcuts: new findings reinforce why nuance is the rule in genetic autoinflammatory syndromes

Paul Tsoukas[a] *and Scott W. Canna*[a,b]

Purpose of review
Practitioners dazed by the evolving concept of autoinflammation are in good company. Despite the clinical challenges autoinflammatory patients present, their study has been fundamental to our understanding of basic human inflammation. This review will focus on the ways in which recent discoveries in genetically mediated autoinflammation broaden and refine the concept.

Recent findings
Major developments in pyrin inflammasome biology, defective ubiquitination, and the hyperferritinemic syndromes will be highlighted.

Summary
We offer a brief discussion of discordance, convergence, genotype, and phenotype in autoinflammation. Additionally, we introduce the concepts of mutation dose effect and hybrid nomenclature. Overall, we hope to provide an update on developments in the field of autoinflammation, some conceptual tools to help navigate the rising tide of discovery, and some encouragement that keeping up with developments in autoinflammation is both exciting and necessary.

Keywords
autoinflammation, inflammasome, interferonopathy, ubiquitination disorders

INTRODUCTION

Autoinflammation was coined to describe illnesses of uncontrolled organ specific and/or systemic inflammation without an apparent infectious or oncologic cause and lacking autoantibody or antigen-specific T-cell responses [1]. Initial discoveries largely revolved around Interleukin (IL)-1 dysregulation. IL-1 inhibition's safety and efficacy in monogenic autoinflammation have paved the way for the study of IL-1 in gout, pericarditis, sepsis, metabolic syndrome, and even thrombosis [2,3,4▪,5,6]. However, the paradigm of 'autoinflammation = IL-1 dysregulation' could not hold for long, and more recent work has implicated several other cytokines and inflammatory signaling pathways.

Single gene defects induce disparate phenotypes and mutations in different genes often converge on classic yet complex phenotypes. It is increasingly clear that there are no shortcuts to understanding their diseases. Our goal is to synthesize the latest discoveries into the existing autoinflammatory landscape. In the interest of space, we will defer exhaustive clinical detail to other excellent and recent reviews [7–10].

CONCISE OVERVIEW OF GENE MUTATIONS AND THEIR CORRESPONDING PHENOTYPES

The following section provides a selected, gene-centric cast of characters, old and new, with relevant clinical and mechanistic updates. Fig. 1

GENE/PROTEIN

Genes implicated in inflammasome disorders

MEFV/PYRIN

Clinical manifestations: the causative gene in the canonical and recessive familial mediterranean fever (FMF), which manifests as recurrent fevers,

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KEY POINTS

- Update on phenotypic diversity, genetic mechanisms, and pathway elucidation of known autoinflammatory genes.
- Divergence, convergence, phenotype, and genotype in autoinflammation.
- Conceptual tools to approach autoinflammatory disease: mutation dose effect and hybrid nomenclature.

erysipeloid rash, arthritis, and serositis [11,12]. Masters *et al.* [13▪] recently identified a dominant syndrome of variable skin disease (acne, abscesses, pyoderma gangrenosum, neutrophilic vasculitis) and prolonged febrile episodes called pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND), caused by specific, highly activating *MEFV* mutations. Recent work suggests that FMF-associated variants, occurring in heterozygosity, may play a role in diverse inflammatory diseases including inflammatory bowel disease (IBD), Behçet syndrome, among others [14,15].

Mechanism: disease-associated MEFV mutations are likely gain-of-function with a substantial gene dose effect (with two alleles required for FMF mutations, but only one for PAAND, see Fig. 2), and cause increased formation of the PYRIN inflammasome. PYRIN is thus, like many other autoinflammatory genes, part of the bacterial sensing machinery [16,17]. This may explain the selection for high carrier frequencies of FMF-associated *MEFV* mutations [9].

NLRP3/NLRP3

Clinical manifestations: NLRP3 (also known as NALP3 or cryopyrin) defects cause the well described spectrum of cyropyrin-associated periodic syndromes (CAPS), including familial cold-induced autoinflammatory syndrome (fever/flu-like symptoms and urticaria), Muckle–Wells syndrome (MWS, + sensorineural hearing loss), and neonatal onset multisystem inflammatory disease (NOMID), + aseptic meningitis, bony articular overgrowth). The lasting and pervasive effects of IL-1 inhibition were recently shown in NOMID, with protection/improvement of all manifestations save bony lesions, including central nervous system (CNS) inflammation [18]. Additionally, anakinra may be more effective in treating/preventing CNS disease in comparison to canakinumab [19].

Mechanism: among the best studied in autoinflammation, NLRP3 responds to various cellular stressors to induce inflammasome assembly. Gain of function mutations (including somatic mutations) can lead to NLRP3 inflammasome hyperactivity and, in particular, excessive IL-1β [10].

MVK/MVK

Clinical manifestations: a spectrum from severe metabolic deficiency to hyper-IgD syndrome (HIDS). Most patients have early-onset fevers, lymphadenopathy, intestinal inflammation, vasculitic skin changes, and arthritis/arthralgia [20,21].

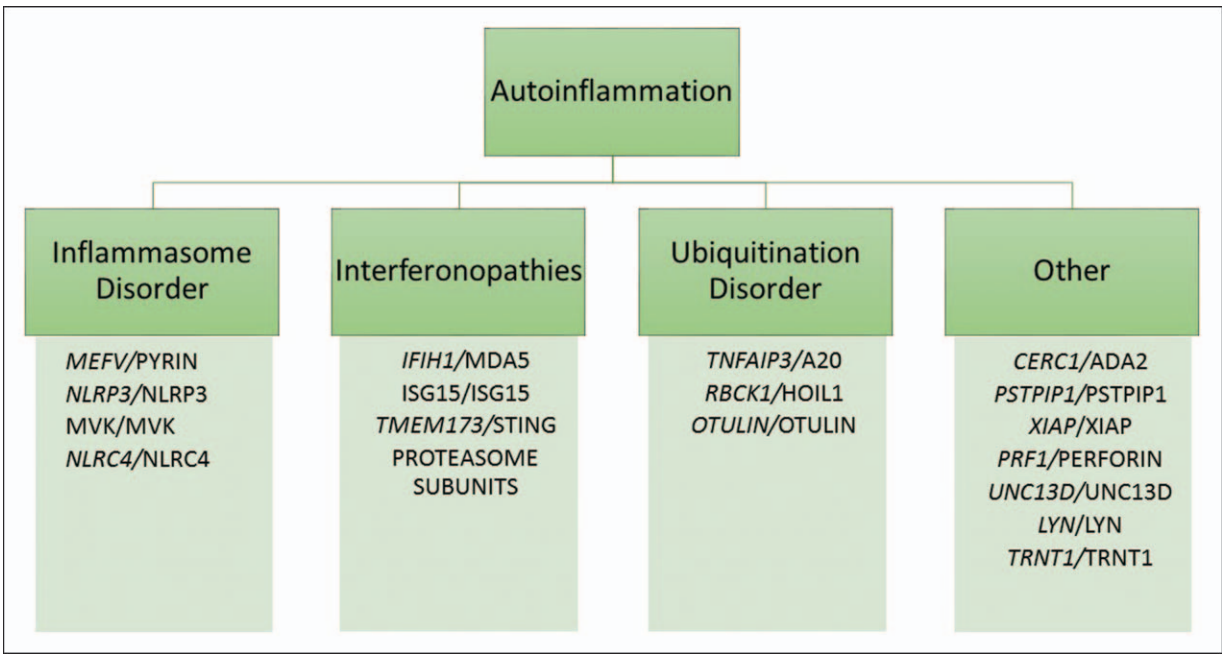

FIGURE 1. Categorization of autoinflammatory disorders based on disease mechanism.

Mechanism: deficiency of the mevalonate kinase enzyme impairs the isoprenoid pathway with effects on cholesterol derivatives, including geranyl groups that serve as protein post-translational modifiers [22]. Recent work has shown that geranylation is a critical part of ρ GTPase activation. Thus, loss-of-function mutations in MVK impair geranylation, decrease ρ-GTPase activity, and thereby increase activity of the PYRIN inflammasome [23▪▪].

NLRC4/NLRC4

Clinical manifestations: gain-of-function NLRC4 mutations were initially described to cause an early-onset syndrome of potentially severe pan enterocolitis, life-threatening macrophage activation syndrome (MAS), and chronic elevation of serum IL-18 [24,25]. Subsequent reports identify a familial urticarial syndrome with less severe systemic inflammation [26,27]. More recent work has expanded the NLRC4-associated spectrum to include NOMID-like phenotypes [28] and in-utero onset of fulminant inflammation and coagulopathy [29▪].

Mechanism: NLRC4 mutations seem to cluster near the ADP-binding site, suggesting this area is important for preventing NLRC4 aggregation and inflammasome activation [24,26–28,30–32]. NLRC4-associated MAS is associated with chronic and profound IL-18 elevation, and IL-18 blocking therapies may be effective [32].

NLRP1/NLRP1

Clinical manifestations: the spectrum of diseases recently associated with NLRP1 mutations lead to varying phenotypes: multiple self-healing palmoplantar carcinomas (MSCP), NLRP1-associated autoinflammation with arthritis and dyskeratosis and familial keratosis lichenoides chronica (NAIAID) [33▪▪,34▪]. Only NAIAID presents as an autoinflammatory condition.

Mechanism: NLRP1 is an inflammasome-activating cytosolic sensor of cytosolic bacterial products like anthrax toxin. As with other syndromes, the mechanisms distinguishing these phenotypes are unknown.

INTERFERONOPATHY-ASSOCIATED GENES

IFIH1/MDA5

Clinical manifestations: neonatal syndrome of inflammation and encephalitis known as Aicardi-Goutieres syndrome. Type 7, unlike other Aicardi-Goutieres syndrome subtypes, can occur after a period of normal development and is associated with widely variable severity. Patients can be minimally symptomatic or have developmental delay/regression, and imaging can identify cerebral atrophy, basal ganglia calcifications, and white matter disease. Some patients present with lupus-like findings (photosensitive vasculitic rash, urticaria, edema), arthritis, serositis, alveolitis, and focal glomerular sclerosis [35]. IFIH1 mutations have also been implicated in systemic lupus erythematosus pathogenesis [36] and a syndrome of early and severe aortic/valvular calcification, dental anomalies, and acroosteolysis: Singleton-Merten syndrome [37].

Mechanisms: gain-of-function mutations in *IFIH1*, which encodes the cytosolic RNA sensor MDA5. Mutations increase MDA5–RNA binding, usually of endogenous ALU retroelements [38].

ISG15/ISG15

Clinical manifestations: severe mycobacterial sepsis following BCG vaccination or minimally symptomatic basal ganglia calcification in unvaccinated children [39,40].

Mechanism: deficiency/loss-of-function mutation. ISG15 is important for mycobacterial-induced interferon (IFN)γ production, but also promotes accumulation of USP18, a potent inhibitor of type I IFN signaling. Thus, ISG15 deficiency leads to both impaired IFNγ production and excessive type I IFN production [40].

PSMB8 and other proteasome subunits

Clinical manifestations: homozygous, digenic, and dominant mutation patterns were recently identified to expand the spectrum of genetic causes for a syndrome of recurrent fevers, anemia, joint contractures, myositis, atypical neutrophilic dermatosis, and lipodystrophy known as chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) or more generally as proteasome-associated autoinflammatory syndromes (PRAAS).

Mechanism: additive loss-of-function defects in PSMA3, PSMB4, PSMB8, PSMB9, and/or POMP may impair proteasome function, driving cell stress and IFN production. IFN signaling puts greater demands on proteasome function, propagating the defect [41–43].

TMEM173/STING

Clinical manifestations: early-onset systemic inflammation, acral vasculopathy, arthritis, myositis, and variable interstitial lung disease known as STING-associated vasculopathy with onset in infancy (SAVI) [44,45]. Janus kinase (JAK) inhibitors

showed preclinical promise, and case reports of ruxolitinib (a selective JAK1/2 inhibitor) in SAVI were promising [46▪]. An ongoing compassionate use study is evaluating the efficacy of JAK inhibitors in various inflammasomopathies, including SAVI (NCT 01724580)

Mechanism: STING links sensing of cytosolic cyclic dinucleotides by cGAS to type I IFN induction, and SAVI mutations cause a gain-of-function, resulting in increased STING activity [47].

UBIQUITINATION DEFECTS DRIVING AUTOINFLAMMATION

TNFAIP3/A20

Clinical manifestations: A20 haploinsufficient patients manifest with early-onset systemic inflammation, and variable features including oral/genital ulcers, uveitis, arthritis, colitis, and cutaneous manifestations like erythema nodosum, pseudofolliculitis, dermal abscesses, and pathergy [48▪▪,49]. The loss-of-function mutation may mimic IBD and Behçet's syndrome, but was also reported to present similarly to autoimmune lymphoproliferative syndrome [50].

Mechanism: A20 is an ubiquitin-modifying enzyme essential in the negative regulation of several important inflammatory pathways including NF-kB signaling, NLRP3 inflammasome activation [51], and NOD signaling [52].

RBCK1/HOIL1

Clinical manifestations: a combination of immunodeficiency and autoinflammation, patients with loss-of-function mutations presented with persistent systemic inflammation associated with amylopectinosis and invasive pyogenic bacterial infections [53].

Mechanism: encodes for HOIL-1, a critical piece of the ternary linear ubiquitination chain assembly complex necessary for normal ubiquitin transfer. Deficiency may decrease NF-kB activity in fibroblasts, but promote IL-1β production in myeloid cells [53].

OTULIN/OTULIN

Clinical manifestations: otulopenia was first described as a syndrome of neonatal onset fevers with neutrophilic dermatosis/panniculitis and failure to thrive without any immunodeficiency [54▪▪,55].

Mechanism: OTULIN is a deubiquitinase, removing the linear polyubitiquitin chain assembled by linear ubiquitination chain assembly complex. Loss-of-function mutations result in OTULIN deficiency and excessive proinflammatory cytokine release downstream of NF-kB, TNF, and NLRP3 pathways [55]. This may be related to selective cleavage of amino-terminal methionine-linked ubiquitin chains that are resistant to disassembly by regulatory ubiquitin modifiers like A20 or CTYLD [56].

UNCLASSIFIED GENES RESULTING IN AUTOINFLAMMATION

CERC1/ADA2

Clinical manifestations: systemic vasculitis resulting in fevers, small-vessel vasculitis akin to polyarteritis nodosa, variable cutaneous manifestations (livedo reticularis, livedo racemose, and Raynaud's to ulcerations and digit necrosis), and, most significantly, early onset ischemic strokes [57,58]. Recent data suggests that all cutaneous polyarteritis nodosa patients should be screened for ADA2 deficiency [59].

Mechanism: deficiency/loss-of-function mutations of ADA2 may cause impaired alternative activation of the vascular macrophages necessary to maintain endothelial cell function/homeostasis [58].

PSTPIP1/PSTPIP1

Clinical manifestations: patients have been described to have some combination of pyogenic arthritis, pyoderma gangrenosum, and cystic acne conglobate (PAPA), hidradenitis suppurativa (lacking arthritis, known as PASH), and seronegative spondyloarthritis (PASS) [60–62]. Fevers are usually not prominent (except with superinfected lesions) and response to cytokine inhibition is variable.

Mechanism: PSTPIP1 (a.k.a., CD2BP1) is a cytoskeletal adaptor. The exact mechanism is unclear, although increased inflammation of macrophages [63] and the pyrin inflammasome [64] have been demonstrated.

X-linked inhibitor of apoptosis/X-linked inhibitor of apoptosis

Clinical manifestations: spectrum of manifestations including features of autoinflammation and immunodeficiency. Classically, X-linked inhibitor of apoptosis (XIAP) deficiency causes a hemophagocytic lymphohistiocytosis (HLH)-like syndrome (splenomegaly, cytopenias, hepatitis, hyperferritinemia) associated with EBV infection [65]. More recently, early-onset/aggressive IBD, hepatitis, recurrent skin infection, periodic fevers, and arthritis have been described [66,67]. Disease may begin *in utero* [68▪].

Mechanism: X-linked deficiency, a loss-of-function mutation, causes an NF-kB induced increase in

apoptosis, necroptosis, and inflammasome activity [69], as well as decreased NOD2 signaling (akin to NOD2-associated Crohn's disease) [70].

PRF1, *UNC13D*, and other genes associated with impaired cytotoxic granules

Clinical manifestations: familial HLH is a well described, typically infantile-onset, life-threatening hyperferritinemic syndrome, often triggered by a viral infection.

Mechanism: though complete deficiency/loss of function of cytotoxicity-associated proteins has been well described to cause familial HLH, a causative role for heterozygous mutations has emerged as contributory in various conditions including systemic juvenile idiopathic arthritis and fulminant influenza sepsis [71]. Such partial cytotoxic defects may prolong immune synapse time and enable excessive cytokine production by cytotoxic cells [72]. Patients bearing digenic defects in cytotoxic genes have also been discovered to develop HLH [73].

LYN/LYN

Clinical manifestations: recent data identify a syndrome of fever and neutrophilic vasculitis associated with B-cell abnormalities, coined LYN-associated autoinflammatory disease [74].

Mechanism: a very specific heterozygous gain-of-function mutation in the LYN tyrosine kinase may result in context-dependent alterations in signaling [75].

TRNT1/TRNT1

Clinical manifestations: TRNT1 deficiency can induce periodic fevers, congenital sideroblastic anemia, B-cell immunodeficiency, and developmental delay (or sideroblastic anemia, immunodeficiency, fevers, and developmental delay, SIFD) [76]. Recently, a hypomorphic TRNT1 variant was associated with retinitis pigmentosa [77].

Mechanism: deficiency/loss-of-function mutation of TRNT1, an RNA polymerase critical for maturation of transfer RNA, may result in cellular stress [78].

INSIGHTS AND CONCEPTUAL FRAMEWORKS INSPIRED BY RECENT DEVELOPMENTS

Convergent phenotypes, many gene

In this section, we focus on the convergence of multiple genetic defects on the classical autoinflammatory phenotypes of urticarial skin rash, IBD, and hyperinflammation. Though the clinical manifestations may be indistinguishable at the bedside, they often result from discrete pathogenic mechanisms and often require different treatments.

Urticaria (NLRP3, NLRP12, PLCG2, NLRC4)

Multiple autoinflammatory diseases share the common characteristic of urticaria. Histologically, the urticaria of an acute allergic reaction is typically characterized by edema and mild eosinophilia. However, in all three CAPS syndromes, it consists of mature neutrophils [79]. Mutations in NLRP12 and PLCG2 also cause cold-induced urticaria, although the histology of these disorders is undocumented [80–82]. By contrast, two large kindreds bearing *NLRC4* mutations were characterized by recurrent episodes of urticaria found to bear a lymphohistiocytic infiltrate [27]. Even though patients with NLRC4-associated urticaria do not appear to develop MAS, the cells in their skin are more characteristic of the infiltrate seen in MAS [32]. Recurrent fever associated with urticaria requires detailed investigation as it is attributable to multiple genetic defects.

Inflammatory bowel disease (XIAP, PLCG2, MEFV, NLRC4, TNFAIP3)

Monogenic early-onset IBD has been associated with defects in innate, adaptive, and barrier immunity [83]. Identification of a genetic cause often has significant therapeutic implications, in particular allogeneic bone marrow transplantation. In addition to defects in IL-10 signaling and regulatory T-cell differentiation, several autoinflammatory genes have been recently implicated. For many genes, IBD may be a 'moonlighting' phenotype, including XIAP deficiency [66,84], MVK deficiency [85,86], PLCγ2 mutations [87], and even MEFV mutations [88]. *NLRC4* mutations associated with MAS also cause a variable, but potentially life-threatening, enterocolitis that may begin *in utero* but appears not to extend beyond early childhood [24,25,29▪]. Finally, although the more common clinical findings of Behçet's syndrome and A20 haploinsufficient patients include mucocutaneous ulceration, a portion of patients experience enterocolitis [48▪▪,89,90].

Hyperinflammation/hyperferritinemia (PRF1, UNC13D, STX11, STXBP2, RAB27A, LYST, XIAP, SH2D1A, NLRC4)

Rather than specific diagnoses, researchers are beginning to consider HLH and MAS as descriptions

of a hyperinflammatory systemic inflammatory response. A variety of genes associated with familial forms of HLH converge on a pathway of impaired cytotoxic granule function. These include *PRF1, UNC13D, STX11, STXBP2, RAB27A, LYST,* among others. Other genes not clearly associated with granule defects can manifest similarly. Deficiency of XIAP or SAP (gene *SH2D1A*) canonically manifests as an EBV-associated HLH-like syndrome [91]. XIAP deficiency, is not associated with cytotoxic impairment. Similarly, the MAS-associated NLRC4 mutations can drive a potentially fatal hyperferritinemic syndrome associated with chronic and extreme elevation of IL-18 but normal cytotoxicity [24,25,32].

SINGLE GENES WITH EXPANSIVE PHENOTYPES

Conversely, mutations within a lone gene can manifest in an array of clinical features. Below are a few examples of genes implicated in driving different autoinflammatory diseases.

NLRP3: Initially, three seemingly disparate syndromes were all associated with inflammasome activing, gain-of-function mutations in *NLRP3.* Now, they constitute the spectrum known as CAPS. To date, almost 200 mutations in the *NLRP3* gene have been documented. Some mutations result in milder phenotypes (R260W) compared with others that are more severe (T348M) [92,93]. Recent findings suggest that NLRP3 mutations with incomplete penetrance promote autoinflammation atypical for CAPS, both phenotypically and in response to IL-1 inhibition [94,95].

MEFV: Implicated as the causative gene in FMF [11,12], only recently have we begun to understand that, akin to CAPS mutations, FMF-associated pyrin mutations are gain-of-function inflammasome inducers [96]. Greater than 300 documented gene variants in MEFV have been described, and of these only a few are canonically associated with FMF. Though FMF is typically inherited in an autosomal recessive pattern, heterozygous FMF has been reported [97]. Recent work by Masters *et al.* also defined a novel monoallelic pyrin-associated inflammasomopathy called PAAND [13▪]. Ordinarily, pyrin is prevented from oligomerizing because it is bound by an inhibitor called 14–3–3. Whereas FMF mutations likely decrease the threshold of stimulus required for pyrin inflammasome activation, PAAND mutations completely disrupt 14–3–3 binding and likely result in a much lower threshold of activation [13▪]. PAAND differs from FMF in that it is characterized by long-lasting recurrent fevers associated with neutrophilic dermatosis, in the absence of serositis and/or amyloidosis. It is, nonetheless, partially colchicine responsive. Thus, FMF and PAAND are two distinct (but similar) clinical entities resulting from different degrees of hyperactivation of the same protein.

In addition to the discovery of PAAND, recent insights into pyrin inflammasome activation shed light on the unique efficacy of colchicine in FMF and the pathogenesis of HIDS. Colchicine likely prevents ρ-GTPase inactivation at the level of the cytoskeleton, thereby preventing the predominant trigger of pyrin inflammasome assembly in FMF [23▪▪]. By contrast, HIDS mutations result in defective geranylation of ρ GTPases themselves, rendering them less active regardless of upstream cytoskeletal stimuli. The suggested pathway also helps expand the role of MEFV. Mutations in B30.2/SPRY domain causing FMF are refractory to colchicine and therefore they may play a role in assembly post-dephosphorylation [23▪▪,96]. Additionally, FMF mutations may activate pyrin inflammasomes independently of microtubules [23▪▪] [98].

NLRP1 and PSTPIP1: variable phenotypes in NLRP1 and PSTPIP1-mediated patients are described above.

NLCR4: Though the initial descriptions were made in 2014, already the gamut of NLRC4-associated autoinflammation runs from mild urticarial to in-utero catastrophe. Clearly, some patients with activating NLRC4 mutations develop early-onset enterocolitis and recurrent, life-threatening MAS [24,25,29▪], but other more classical autoinflammatory phenotypes have also been associated with NLRC4 hyperactivity. A familial urticaria has been described, potentially with a low incidence of enterocolitis [26,27], as well as a NOMID-like state with CNS inflammation [28]. Phenotypes differ even in kin with identical mutations [24,26]. Response to treatment varies as well; some patients requiring minimal therapy [27], others with variable responses to IL-1 inhibition, and some refractory to multiple modalities that may benefit from inhibition of IL-18 [32]. Although pathogenic NLRC4 mutations all seem to cluster near the nucleotide-binding pocket, and some areas track with disease (amino acids 337–341 and MAS, 443–445 and urticaria), there are not yet functional correlates to these observations.

XIAP: XIAP deficiency is now known to cause a remarkable diversity of inflammatory phenotypes, spanning the distinction between immunodeficiency and autoinflammation. Deficiency of XIAP clearly drives an X-linked HLH-like syndrome particularly in response to EBV [65,91]. However, expanded genetic diagnostics have uncovered XIAP

deficiency as a cause of early onset IBD or Mendelian IBD [66,84,99]. Other investigators have associated recurrent skin abscesses, periodic fevers, and arthritis [67]. There are some similarities between NLRC4-associated MAS and XIAP deficiency: both can induce severe early-onset IBD and recurrent MAS/HLH flares, both have been associated with in-utero autoinflammation [68▪], and both have outsize upregulation of IL-18 [100]. Consistent with its confusing spectrum of disease, the mechanisms by which XIAP causes disease include abnormalities in cell death, NOD2 signaling, and inflammasome activation [69].

COMPLEX GENETICS AND THE MUTATION DOSE EFFECT

Mosaicism: Mosaicism refers to mutation(s) in only a subset of an individual's cells. With the progress of deep sequencing, it is likely that we will discover more mosaic mechanisms of genetic pathogenesis. Mosaicism may explain the later onset of classically autoinflammatory symptoms, particularly if the mutation confers a survival or proliferation benefit [101,102]. Mosaicism of NLRP3 has been best described in 'mutation-negative' CAPS [103–105], but has more recently been associated with Schnitzler syndrome [102]. The discovery of gonosomal mosaicism is important for genetic counseling in both symptomatic Muckle–Wells syndrome and asymptomatic parents [106]. Pediatric granulomatous arthritis can be caused by somatic [107] and gonosomal mosaicism [108] of NOD2. Similarly, somatic NLRC4 mutations have caused both a NOMID-like phenotype and in-utero fatal hyperinflammation [28,29▪]. Importantly, these are all examples of somatic gain-of-function mutations in inflammasome activators. This may be related to the ability of inflammasomes to 'jump' between cells and thereby propagate inflammation from a mutant to a nonmutant macrophage [109].

Digenic Inheritance: In addition to the multigenic mechanisms that likely contribute to diseases like sJIA and Behçet's, digenic modes of inheritance have been demonstrated. Such mechanisms may account for variable disease penetrance or earlier/more severe disease than might be expected with either mutation alone [110]. Additive or synergistic mutational combinations, including NLRP3, MEFV, and TNFRSF1A, can lead to FMF-like phenotypes [111]. Recurrent fever and chronic aseptic meningitis was thought to be because of the combined effects of TNFRSF1A and MEFV mutations [112]. Similarly, monoallelic mutations in different proteasome subunits (in addition to PSMB8) have been shown to drive PRAAS/CANDLE like disease [43]. Digenic mechanisms of cytotoxic dysfunction have also been described in some familial HLH patients, with synergistic heterozygous mutations in complementary cytotoxic granule-associated pathways (e.g., PRF1 and UNC13D) [73].

Mutation dose: We do our best to characterize each mutation as gain or loss-of-function. However, we can now appreciate that each mutation may be associated with a specific pathogenic potency. Recent developments as described above and illustrated below, highlight that differences in the potency of a mutation may matter as much as the gene affected. Thus, on a per allele basis, phenotype can vary drastically with the potency of a given mutation (Fig. 2). Consistent with this idea, the mutation dose effect is a concept that may help in understanding several new genetic observations of biologic and clinical importance.

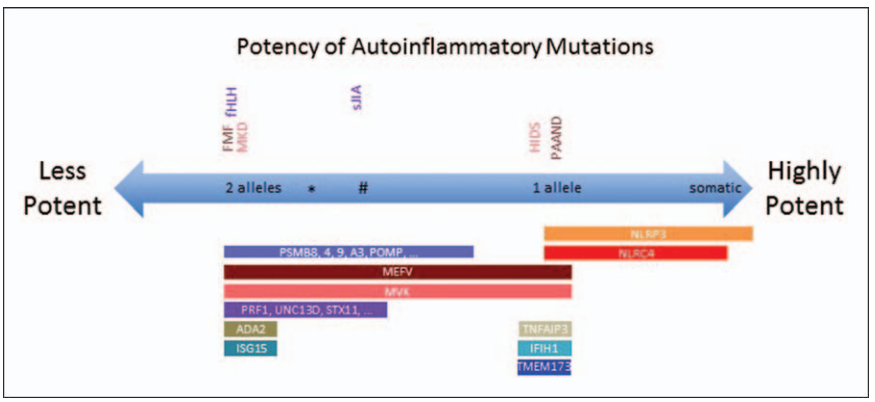

FIGURE 2. Schematic representation of known potency of disease-associated mutations. IL-1/inflammasome-associated diseases are in lighter grey tones, IFN-associated diseases in darker grey tones. [a]Digenic mechanisms that apply to cytotoxicity-associated and proteasome-associated gene defects. [b]Heterozygosity may confer an increased risk.

CONCLUSION

Few fields have witnessed such a dramatic, patient-driven sequence of rapid discoveries. Autoinflammatory patients do not simply illustrate established pathways. Instead, the process of linking patient mutations, clinical phenotypes, mechanistic insights, and often responses to treatment, has been critical to defining basic inflammatory pathways. The way forward is equal parts daunting and exciting. Clinicians must expand our perspectives to include an increasing number of genes and pathways, how specific mutations impair function, and how multiple mutations may interact.

These new findings also highlight a looming quandary of nomenclature. Some diseases with multiple genetic causes are still identified by phenotype (e.g., IBD, MAS/HLH), although others with varying phenotypes are identified by a specific gene (e.g., CAPS and PRAAS). Though the rationale for choosing has been largely historical to date, our opinion is that gene centric or hybrid (e.g., NLRC4-MAS vs NLRC4-urticaria) approaches are preferable.

The pantheon of monogenic autoinflammatory diseases will continue to grow, adding genes not previously associated with autoinflammation as well as nuance and depth to old favorites. Centola *et al.*'s [1] prophecy 20 years ago resonates more than ever; 'the molecular characterization of the periodic fever genes should provide important new insights into the regulation of inflammation in general.' It is both our privilege and responsibility to further that goal.

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Conflicts of interest

There are no conflicts of interest.

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The catastrophic antiphospholipid syndrome in children

Ellen J.L. Go and Kathleen M. O'Neil

Purpose of review
To review the difficult syndrome of catastrophic antiphospholipid syndrome, emphasizing new developments in the diagnosis, pathogenesis and treatment.

Recent findings
Few recent publications directly address pediatric catastrophic antiphospholipid syndrome (CAPS). Most articles are case reports or are data from adult and pediatric registries. The major factors contributing to most pediatric catastrophic antiphospholipid syndrome include infection and the presence of antiphospholipid antibodies, but complement activation also is important in creating diffuse thrombosis in the microcirculation. Treatment of the acute emergency requires anticoagulation, suppression of the hyperinflammatory state and elimination of the triggering infection. Inhibition of complement activation appears to improve outcome in limited studies, and suppression of antiphospholipid antibody formation may be important in long-term management.

Summary
CAPS, an antibody-mediated diffuse thrombotic disease of microvasculature, is rare in childhood but has high mortality (33–50%). It requires prompt recognition and aggressive multimodality treatment, including anticoagulation, anti-inflammatory therapy and elimination of inciting infection and pathogenic autoantibodies.

Keywords
antiphospholipid antibody, catastrophic antiphospholipid syndrome, complement, microangiopathy, thrombosis

INTRODUCTION

Catastrophic antiphospholipid syndrome (CAPS) is a disease characterized by rapid development of thromboses in several organs leading to their dysfunction and failure, in the presence of antiphospholipid antibodies. The preliminary classification criteria proposed by the International Task Force in 2003 considers patients to have definite or probable CAPS based on the number of criteria met [1].

The classification scheme (Table 1) was validated 2 years later demonstrating good sensitivity (90.3%), specificity (99.4%), positive predictive value (99.4%) and negative predictive value (91.1%) [2▪]. Although these criteria were not intended for diagnosis, they are often used as such because there are no other validated criteria for identifying this syndrome. Early recognition is important, as CAPS has a high mortality rate of 33–50% [3,4]. Because it is rare and challenging to diagnose, advances in understanding of pathogenesis, diagnosis and best treatment have been slow. Here, we will highlight points that distinguish CAPS from typical antiphospholipid syndrome (APS) and emphasize pediatric considerations in management.

CAPS is a distinct entity and it differs from severe APS that has a devastating outcome. Both conditions are accompanied by antiphospholipid (aPL) antibodies and represent the clinical consequences of abnormal thrombotic control. However, they vary in the type of vessels involved; this is perhaps the most important distinguishing feature. Untreated APS can occur in any organ system and

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KEY POINTS

- The CAPS is a rare cause of autoantibody-mediated diffuse microvascular thrombosis and multiorgan failure in childhood.
- Because of high mortality (33–50%), early recognition and aggressive treatment are required.
- Combination therapy aimed to treat underlying triggers (often infection in children), suppress inflammation and block life-threatening disseminated thrombosis is needed.
- Inhibition of complement activation and suppression of autoantibody production may prove important treatment adjuncts.
- Collaborative clinical research is needed in this rare but potentially devastating pediatric disorder.

lead to permanent disability, severe morbidity or even death due to occlusion of single or several medium or large blood vessels. In contrast, the characteristic feature of CAPS is precipitous, widespread microvascular occlusion. Its pathology resembles other thrombotic microangiopathic disorders like disseminated intravascular coagulation and thrombotic thrombocytopenic purpura (Fig. 1) [5]. Another hallmark is the evidence of the systemic inflammatory response syndrome (SIRS) in CAPS which is not seen in the more typical APS.

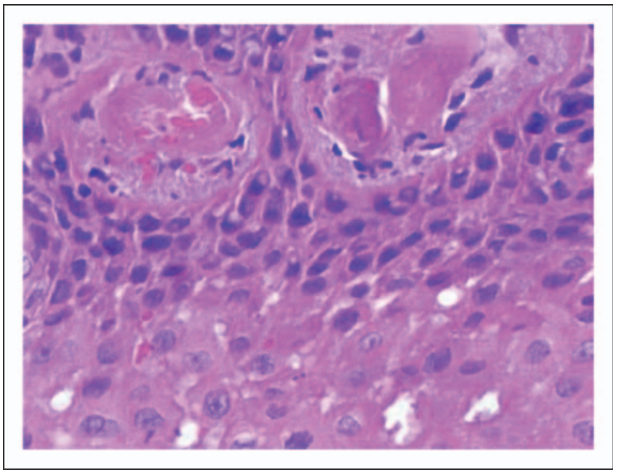

FIGURE 1. Catastrophic antiphospholipid syndrome histopathology. The histopathology of a skin biopsy from the finger of a child with catastrophic antiphospholipid syndrome demonstrates numerous small vessel thromboses in capillaries without evidence of vessel wall inflammation. Reproduced with permission from [5].

Table 1. Diagnostic criteria for catastrophic antiphospholipid syndrome[a]

Diagnostic criteria for CAPS
Evidence of involvement of 3 or more organs, systems and/or tissues
Development of manifestations simultaneously or within 1 week
Laboratory confirmation of antiphospholipid antibodies (lupus anticoagulant and/or anticardiolipin and/or anti-β2-glycoprotein I antibodies in titers higher than 40 IU/l)
Exclusion of other diagnoses
Definite CAPS
All four criteria are present
Probable CAPS
All four criteria but only two organs, systems or tissues involved
All four criteria but laboratory confirmation at least 6 weeks apart is not available due to early death or no testing for APL prior to onset of CAPS
Presence of criteria 1, 2 and 4
Presence of criteria 1, 3 and 4, and the development of a third thromboembolic event despite anticoagulation treatment more than 1 week but less than 1 month after the second event

APL, antiphospholipid antibody; CAPS, catastrophic antiphospholipid syndrome.
[a]Adapted with permission [22].

PATHOPHYSIOLOGY

There are multiple mechanisms involved in CAPS pathophysiology including: molecular mimicry [6], excessive cytokine release [7▪], thrombotic storm [8] and endothelial activation and/or complement activation [9]. Briefly, microbial products and lipopolysaccharides bind to Toll-like receptor 4 (TLR 4) generating intracellular signals that lead to NF-kB activation and release of proinflammatory cytokines [10]. Existing microthrombi promote an antifibrinolytic state through an increase in plasminogen activator inhibitor and depression of procoagulant factors, creating an imbalance in homeostasis of the coagulation system favoring further thrombus formation. Anti-β2-glycoprotein I (anti-β_2-GPI) antibodies directly induce endothelial cell activation via the MyD88 pathway and suggest a possible association between β_2-GPI and members of the TLR 4 family [11,12]. Complement (C) activation products C3a and C5a, attract neutrophils, and complement activation itself activates the coagulation cascade directly through complement serine proteases cleaving prothrombin to thrombin, and via TLR pathways. Complement activation also mediates release of inflammatory cytokines [13▪]. The serine proteases belonging to the coagulation system are able to activate the complement cascade independently, and vice versa. It is important to emphasize the cross-talk between these two distinct

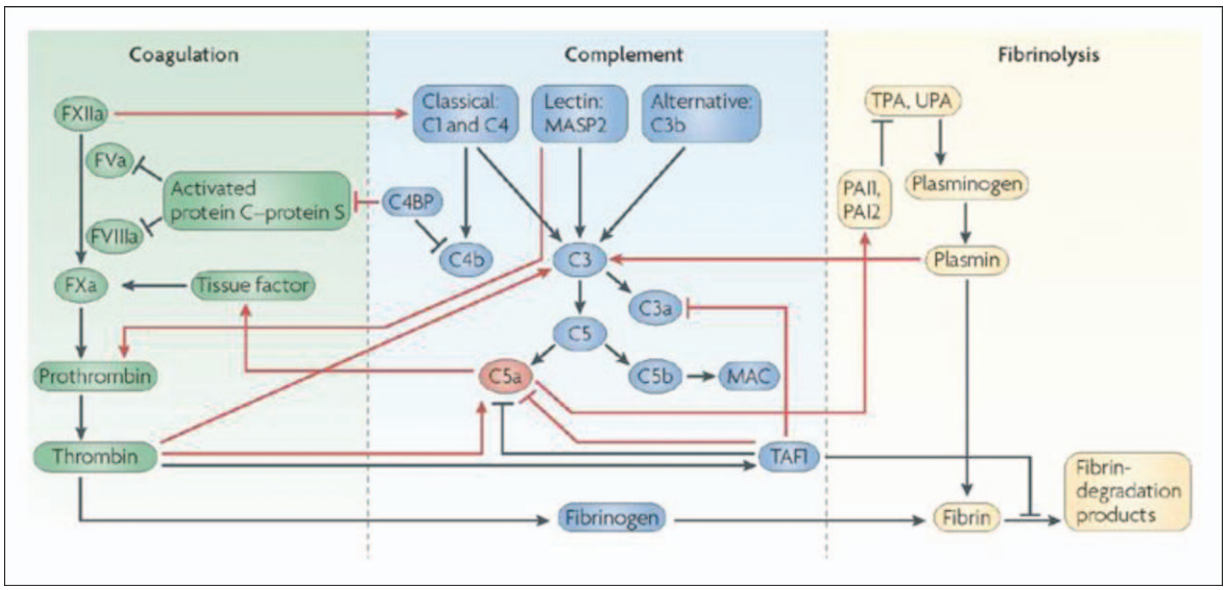

FIGURE 2. Interaction of the complement and coagulation cascades. This figure illustrates the interactions among serine proteases and their inhibitors in the coagulation, complement and fibrinolytic cascades. Note that not only can complement enzymes cleave prothrombin to thrombin and thrombin can cleave C3 to the active C5 convertase enzyme, but C4 binding protein binds C4 and the anticoagulant, protein S. Low levels of C4 in immune complex disease make more C4BP available to bind protein S, limiting its anticoagulant activity. Reproduced with permission from [14].

but interconnected systems in terms of treatment consideration for CAPS (Fig. 2, [14]).

MANIFESTATIONS

CAPS occurs in fewer than 1% of patients with APS and juvenile CAPS is exceedingly rare, representing only a small portion of overall CAPS patients [15]. Most of what is known about the clinical characteristics, manifestations and prognosis of pediatric CAPS comes from the International CAPS registry. They have 60 cases of CAPS with onset of catastrophic event below 18 years of age included in their recent descriptive analysis [16▪]. Most were girls (67%) and had no underlying autoimmune disease (59%). Infection was the most common precipitating factor across all age groups, but especially in children (54%). Malignancies are more frequent triggers in elderly patients (33 vs. 14%, $P < 0.001$). CAPS was the first manifestation of APS in 86% of pediatric patients, compared with 44% in patients 18 years and older. Extensive parenchymal organ involvement is predominant in CAPS but not in APS. Renal, pulmonary, cardiac and central nervous systems are most affected. Peripheral disease was characterized by venous thrombosis (37 vs. 23%, $P = 0.015$) in children in contrast to arterial thrombosis in elderly patients (33 vs. 16%; $P = 0.03$). Defreitas *et al.* [17▪▪] reviewed and summarized 21 published cases of pediatric CAPS from 1992 to 2014. The youngest patient was 3 months old (mean age 10.5 ± 4.8 years; median = 12 years). Fourteen (66%) were girls and 16 (76%) had no prior rheumatic disease. There was an infectious trigger in 13 (62%), two followed surgery, three were secondary to systemic lupus erythematosus exacerbation without infection, one had CAPS as the primary manifestation of a malignancy and two had no known trigger.

Among 140 children with APS included in the Ped-APS Registry, 49% had an underlying autoimmune disease vs. just 24% in CAPS. Thrombotic events in the Ped-APS Registry included venous thrombosis 61%, arterial thrombosis in 31%, small vessel thrombosis in 5% and mixed arterial and venous thrombosis in 3% of patients [18▪▪].

LABORATORY FINDINGS

Laboratory investigations in the CAPS registry showed that anti-β2-GPI IgG, lupus anticoagulant (LAC) and anticardiolipin (aCL) IgG antibodies were the most often implicated aPL antibodies (91, 79 and 78%, respectively) in children. Adult and elderly patients show similar aPL profiles, although LAC is more commonly found in adults. It is important to take note that some routinely used ELISA for anti-β2-GPI antibody lack standardization, agreement on cutoff values and has wide inter-assay and intra-assay variation [19,20]. Each aPL test also differs in sensitivity and specificity; aCL antibodies have higher diagnostic sensitivity, whereas anti-β2-GPI

antibodies are more specific. Thus, anti-β2-GPI is able to detect nonpathogenic antibodies and phospholipid-independent anti-β2-GPI antibodies, making them less suitable as a general diagnostic test. Two LAC tests with different assay principles reduce the likelihood of obtaining false-positive results [21]. The recommended assays include the dilute Russell viper venom test, the silica clotting time and the lupus sensitive aPTT-based (activated Partial Thromboplastin Time) method. LAC is more strongly associated with thrombosis and clinically adverse findings in APS [22]. Functional assays are more predictive of thrombotic risk than the antibody assays. A major limitation of current laboratory diagnosis is that one cannot reliably test LAC in patients receiving anticoagulants. Many other phospholipid antibodies are described, but further studies are needed to determine their significance in CAPS.

TREATMENT

No pharmacologic agent has been approved by the United States Food and Drug Administration for treatment of CAPS in childhood. Early disease recognition with prompt initiation of aggressive therapy when CAPS is suspected is felt to improve patient survival. The Task Force on CAPS recommends anticoagulation and corticosteroids as the initial approach [23▪]. Treatment should be aimed at removing the triggering factor if known, controlling SIRS and eliminating existing thrombus.

Anticoagulation with heparins is considered the mainstay of therapy not only for its thrombolytic and fibrinolytic properties but also its inhibitory effect on complement activation [24]. This is reflected in the registry where 82% received heparin anticoagulation either alone or combined with other agents. Despite its widespread clinical use, anticoagulation therapy remains a challenging therapeutic area in pediatrics. One must consider developmental hemostasis and that distribution, binding, clearance and pharmacodynamic responses of antithrombotic drugs are age-dependent [25,26]. This makes drug dosing, monitoring and interaction in children different from adults and ultimately affects a patient's response to therapeutic agents. Neonates have increased drug clearance, reduced antithrombin levels and lesser capacity to generate thrombin, which results in relative heparin resistance [25]. They also have higher baseline aPTT and this affects therapeutic monitoring of unfractionated heparin [27,28]. Weight-based dosing may not result in equivalent anticoagulant effect in infants and children of different ages; therefore, diligent monitoring and dose adjustments are needed [29]. Acutely, heparin is the anticoagulation treatment of choice because of its rapid onset as well.

Long-term anticoagulation with either oral warfarin or low-molecular-weight-heparin (LMWH) generally depends on practical issues like ease of administration, level of comfort and monitoring required. Subcutaneous LMWH has lower risk of heparin-induced thrombocytopenia than unfractionated heparin, needs less monitoring and has fewer drug interactions, but is more costly. It has been shown that younger children may not achieve therapeutic anticoagulation when using standard doses of LMWH and require dose changes to achieve the desired anti-Factor Xa range [30▪]. This is attributed to greater volume of distribution, faster drug clearance and ontogeny. Warfarin is available as a tablet which makes it more acceptable to children compared with chronic LMWH that is injected. Challenges of prescribing warfarin include difficulty in achieving a stable drug level in relation to diet variations, drug–drug and drug–alcohol interactions, and the impact of genetic polymorphisms (CYP2C9 and VKORC1) on warfarin dose requirements [31]. Risks and benefits should be carefully considered when deciding on which anticoagulation to use for maximum efficacy and least adverse outcomes. Warfarin does not inhibit complement activation, moreover.

Since CAPS appears to be fueled by inflammation and its effects on endothelium, it is important to control inflammation to halt the process. Glucocorticoids decrease platelet aggregation, endothelial and leukocyte adhesion, production of plasma-derived (C3, C5a, bradykinin, thrombin) and cell-derived (cytokines, nitric oxide) inflammatory mediators. Steroids also reduce transcription of proinflammatory genes via inhibition of nuclear translocation factor NF-kB [32]. Glucocorticoids also upregulate anti-inflammatory processes like phagocytosis, chemokinesis and antioxidative processes, all of which are beneficial in minimizing SIRS. Seventy-six percent of 522 episodes in the CAPS registry were treated with corticosteroids as part of a combination regimen; however, when used alone, glucocorticoids resulted in lower rate of recovery and poor prognosis, and thus, this is not recommended [3]. *In vitro*, however, steroids can directly activate coagulation and inhibit fibrinolysis [33]. There is strong evidence of the association of glucocorticoids treatment with venous thromboembolic events. This should not discourage the use of steroids when indicated, but we urge providers to be cautious when deciding on the dose and duration of glucocorticoid treatment. One should decrease the dose as soon as feasible if the trigger to

SIRS has resolved, and consider steroid-sparing anti-inflammatory treatment early in the management of CAPS.

Therapeutic plasma exchange (TPE) is the third most commonly used treatment modality in CAPS. It effectively removes pathologic antibodies, immune complexes and cytokines [34]. TPE is a generally well tolerated and effective option; however, the technical considerations in younger pediatric patients (obtaining adequate vascular access, removing large volumes of plasma and giving adequate replacement fluid) can be a challenge. Children have a higher TPE-associated complication rate (55% of procedures, and 82% of patients) compared with adults and so they should be admitted in specialized care centers where this is done regularly [35,36]. Delay in starting treatment in other microangiopathic disorders, like thrombotic thrombocytopenic pupura, is a strong predictor of poorer outcome. In our opinion, TPE must be considered early in CAPS.

Intravenous immunoglobulin (IVIg) modulates complement activation, neutralizing C3a and C5a. It inactivates immune complexes, neutralizes binding of aCL to cardiolipin, inhibits LAC activity and decreases antibody production by inactivating B cell clones [37–40]. High-dose IVIg at doses of 2 g/kg is used a part of the 'triple therapy' in CAPS and should be given after TPE for maximum benefit. Aside from the mild, reversible IVIg-related acute reactions like headache, fever and myalgia, administration is also associated with serious thromboembolic events. The thrombogenic potential of this agent is attributed to blood stasis with hyperviscosity from high levels of IgG, immune complex formation, and increased platelet aggregation [41]. In CAPS patients, it is advisable to give IVIg at lower concentration and slow infusion rate. In cases of renal impairment, sucrose-free preparations are preferred due to nephrotoxic potential of sucrose [42].

OTHER AGENTS

Evidence of effective use of rituximab comes from a phase II pilot study that looked at safety of rituximab in 19 adult APS (not CAPS) patients [43▪]. The study showed that rituximab is well tolerated in aPL-positive patients and may be effective in controlling some but not all noncriteria manifestations of APS, even though it did not change the patient's aPL profiles. In refractory CAPS, anti-B-cell therapy has been tried with success in very few case reports and case series [44,45▪]. There were a total of 20 patients in the CAPS registry report in 2013 who received rituximab either as a first-line drug (40%) in combination with triple therapy or as a second-line agent (60%); none of them are children. After 9.5-month median follow-up, 16 patients survived and there were four deaths. Except for the two patients who required at least a second course of rituximab because of recurrent thrombosis, none of the surviving patients developed thrombosis after treatment. In Defreitas's pediatric CAPS review, all four patients who got rituximab recovered. No definite conclusion regarding rituximab's efficacy in CAPS can be made at present. However, with very limited therapeutic options in a life-threatening situation, rituximab may still have a role in the long-term management of catastrophic APS patients.

Eculizumab is a humanized mAb against complement C5 that inhibits formation of C5a. Terminal complement blockade prevents formation of aPL-induced thrombosis in mouse models [46,47], and the clinical benefit of eculizumab in patients with APS highlights the importance of targeting complement inhibition in disease pathogenesis in CAPS. Indeed, it has been used successfully in a limited number of patients with CAPS, showing improvement in thrombocytopenia and clinical status [48–50,51▪▪,52]. Significantly, all patients who were treated with eculizumab had recurrent thrombosis despite rituximab therapy. Eculizumab is a promising alternative and should be considered earlier in refractory CAPS as treatment and thrombus preventive strategy.

There is not enough evidence for definite role of immunosuppression in CAPS. In pediatric CAPS at least, patients who received immune suppression were four times more likely to survive than those who did not, but each treatment regimen failed to reach statistical significance because of small sample size [17▪▪].

CONCLUSION

CAPS is one of the most dangerous and perplexing autoantibody defined processes in childhood. Widespread microcirculatory thrombosis even without presence of detectable antiphospholipid antibodies should raise suspicion for this diagnosis. Once CAPS is considered, one ought to look for a potential infectious trigger and assume this to be present at least until proven otherwise. Medical management must be optimized to promote maximum efficacy and limit adverse effects and toxicity in pediatric patients. Current consensus in therapy is to start anticoagulation and corticosteroids with or without plasma exchange and/or IVIg. Biologics have been used successfully in children with refractory CAPS, but larger studies are needed. The international CAPS registry is an important resource in helping

us to understand the disease and monitor outcomes of these patients. However, there is a need to have a separate registry that focuses on learning about cause, mechanisms, natural history, treatment and impact on quality of life children with CAPS.

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Conflicts of interest

K.M.O. has received honoraria for consultation from Eli Lilly. E.J.L.G. has none to declare.

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Update on the pathogenesis and treatment of juvenile idiopathic arthritis

Gabriella Giancane, Alessandra Alongi, and Angelo Ravelli

Purpose of review
To provide an overview of recently published studies on pathogenesis and management of juvenile idiopathic arthritis (JIA).

Recent findings
In the past year, the potential role of network analysis in the understanding of the molecular phenotype of individual JIA subgroups has been highlighted. In addition, potential new targets for pharmacologic interventions have been identified through the elucidation of mechanisms that modulate the function of cells involved in the inflammatory process. There is a growing interest for the role of the gut microbiome in disease pathogenesis, which may open the way to future therapeutic manipulations of fecal microbial population. Recent therapeutic studies have provided important information in large patient samples on the effectiveness and toxicity profile of biologic medications used in JIA. Concomitant administration of methotrexate was found to increase the effectiveness of intra-articular corticosteroid therapy in children with oligoarticular JIA.

Summary
A great deal of work is being conducted to better define the molecular phenotype of the individual subsets of JIA and to identify potential new targets for therapeutic interventions. The results of the ongoing large-scale international data collections will help establish the long-term safety profiles of biologic medications, in particular the risk of malignancy.

Keywords
juvenile idiopathic arthritis, pathogenesis, treatment

INTRODUCTION

The etiologic factors and pathogenesis of juvenile idiopathic arthritis (JIA) are still elusive. It is hypothesized that a genetically susceptible individual could develop an uncontrolled and harmful immune response towards a self-antigen after exposure to an unknown environmental trigger [1]. However, pathogenetic investigations should take into account that JIA is not a single disease, but constitutes a heterogeneous group of illnesses with presumably distinct genetic background and pathophysiology [2] (Table 1).

In the last two decades, the management of JIA has been revolutionized by the introduction of biologic response modifiers, which have provided an effective therapeutic option for the treatment of patients who are resistant to conventional antirheumatic medications, namely methotrexate (MTX) or sulfasalazine [3] (Table 2). These advances have increased the expectation for disease control [4]. In addition, evidence is accumulating to support the benefit of early aggressive therapy [5,6].

In this review, we provide an update of studies published in the last year regarding pathogenesis and treatment of JIA.

UPDATE ON PATHOGENESIS

Systems biology, conducted through the analysis of networks of genes and proteins, is a powerful tool to identify biological pathways of relevance to complex genetic diseases. Application of network biology can provide new insights into the molecular heterogeneity of JIA and help to refine its classification. Network analysis of transcriptomic datasets

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KEY POINTS

- Network analysis is a powerful new tool to define of the molecular phenotype of individual JIA subgroups.
- There is growing interest for the pathogenetic role of the gut microbiome and the therapeutic potential of the manipulation of fecal microbial population.
- Large-scale therapeutic studies have confirmed the sustained efficacy and acceptable safety profile of biologic medications in the different subsets of JIA.
- A controlled trial suggested that the combination with methotrexate may increase the effectiveness of intra-articular corticosteroid therapy in children with oligoarticular JIA.

revealed different gene expression profiles between children with early (<6 years) or late (≥6 years) disease onset [7▪▪]. The observed correlation between age and variability in gene expression supports the proposal to incorporate the age at disease onset among classification criteria [8,9].

The increased prevalence of autoimmunity among first-degree relatives of patients with JIA, recently shown by data from the CARRA Registry [10], together with the previous reports of familial aggregation of JIA and of monozygotic twin concordance rate of 25–40% [11], suggests that genetic factors play a major role in immunopathogenesis. The major histocompatibility complex has long been recognized as the most important contributor to JIA susceptibility. A genotyping analysis from this region revealed a strong association between human leukocyte antigen (HLA)-DRB1 amino acid position 13 and both oligoarthritis and rheumatoid factor-negative polyarthritis [12▪]. The shared genetic association of these two subtypes is in keeping with the notion that in the current JIA classification patients with homogeneous features are misplaced in diverse disease categories [13,14]. The observation in the same study of mixed HLA association

Table 1. International League of Associations for Rheumatology classification criteria for juvenile idiopathic arthritis in childhood

ILAR category	%	Definition	Exclusion criteria
Systemic arthritis	4–17	Arthritis with or preceded by daily fever of at least a 2-weeks, quotidian for at least 3 consecutive days + one or more among: Evanescent nonfixed erythematous rash Generalized lymph node enlargement Hepatomegaly and/or splenomegaly Serositis	a, b, c, d
Oligoarthrtitis	27–60	Arthritis of 1–4 joints within the first 6 mo	a, b, c, d, e
Persistent	40	≤4 joints affected during the whole disease course	
Extended	20	>4 joints affected after 6 mo of the disease	
Polyarthritis RF-negative	11–30	Arthritis of >4 joints within the first 6 mo (RF−)	a, b, c, d, e
Polyarthritis RF-positive	2–7	Arthritis of >4 joints within the first 6 mo (RF+)	a, b, c, e
Psoriatic arthritis	2–11	Arthritis and psoriasis or arthritis + at least two among: Dactylitis Nail abnormalities (pitting or onycholysis) Psoriasis in a first-degree relative	b, c, d, e
Enthesitis-related arthritis	1–11	Arthritis and enthesitis or one of them + at least two among: Presence or history of sacroiliac joint tenderness and/or inflammatory lumbosacral pain HLA-B27+ Onset of arthritis in a male older than 6 yrs Acute symptomatic anterior uveitis History of ankylosing spondylitis, enthesitis-related arthritis, sacroiliitis with inflammatory bowel disease, Reiter syndrome or acute anterior uveitis in a first-degree relative	a, d, e
Undifferentiated arthritis	11–21	Criteria in no category or two or more of the above categories	

Exclusion criteria: a: Psoriasis or a history of psoriasis in the patient or a first-degree relative; b: Arthritis in a HLA-B27 positive male beginning after 6 years of age; c: ankylosing spondylitis, enthesitis-related arthritis, sacroiliitis with inflammatory bowel disease, Reiter syndrome or acute anterior uveitis or a history of one of these criteria in a first-degree relative; d: presence of IgM RF positive on at least two occasions at least 3 months apart; e: presence of systemic JIA.
ILAR, International League of Associations for Rheumatology; JIA, juvenile idiopathic arthritis; mo, months; RF, rheumatoid factor; yrs, years.

Table 2. Main characteristics of biologic agents currently used in juvenile idiopathic arthritis

Drug	Molecule	Target	Dosage	Route	Half life
Etanercept	TNFRII/FcIgG1	TNF-α,β	0.8 mg/kg/week or 0.4 mg/kg twice weekly; maximum dose: 50 mg	sc	102 ± 30 h
Adalimumab	mAb anti-TNF-α	TNF-α	24 mg/m2 q2 weeks; max 40 mg	sc	10–20 days
Infliximab	mAb anti-TNF-α	TNF-α	6–10 mg/kg q2 weeks – 2 months	iv	200 h
Abatacept	CTLA4-Ig	CD80/86	10 mg/kg week 0,2,4, then q4 weeks	iv sc	13.1–14.3 days
Tocilizumab	mAb anti IL-6R	IL-6	Poly JIA >2 yrs, <30 kg 10 mg/kg q4 weeks Poly JIA >2yrs, >30 kg 8 mg/kg q4 weeks sJIA >2 yrs, <30 kg 12 mg/kg q2 weeks >2 yrs, >30 kg 8 mg/kg q2 weeks	iv	8–14 days
Anakinra	IL1-Ra	IL-1α,β	1–4 mg/kg/day	sc	4–6 h
Canakinumab	mAb anti IL-1	IL-1β	≥2 years: 4 mg/kg/dose q 4 weeks Maximum dose: 300 mg	sc	23–26 days
Rituximab	mAb anti CD20	B cells	375–500 mg/m^2 q week x 2 doses up t o 1 g adult dose	iv	30–400 h

IL, interleukin; iv, intravenous; Poly, polyarticular; sc, subcutaneous; Syst, systemic; tbc, tuberculosis; yrs, years.

in juvenile psoriatic arthritis patients argues against the assumptions that children with this condition constitute a single homogeneous population [15▪].

A large association study on European patients revealed that HLA-DRB1*11 alleles, which are linked to oligoarticular and rheumatoid factor-negative polyarticular JIA, confer an increased risk of developing systemic JIA [16]. Note that by demonstrating that class II HLA molecules influence disease susceptibility, this study implicates adaptive immunity in the pathogenesis of systemic JIA. This finding is a bit surprising, given the well established prominent role of the innate immune system in this disease, which has led to propose its inclusion in the autoinflammatory disease spectrum[8]. However, the clinical and biologic distinction between systemic arthritis and the other JIA subtypes was emphasized by a subsequent genome-wide association study, which showed that systemic disease bears a unique genetic architecture [17▪].

Macaubas *et al.* [18] found impaired response of systemic JIA monocytes to interferon (IFN). The responsiveness was, however, restored after a change in the medication regimen, particularly the introduction of a biologic agent. Impaired IFN/STAT1 signaling suggested a skewing of monocytes toward a regulatory M2 phenotype, which may underlie disease pathophysiology. Regulatory macrophage polarization in systemic JIA was also found to be driven by the microRNA-125a-5p. The expression of this microRNA on monocytes was significantly elevated in patients with active disease, ongoing systemic features and increased acute phase reactants [19]. Altogether, these studies draw attention to potential new targets for therapies aimed at modulating the function of monocytes and macrophages implicated in the inflammatory process.

The phenotype and functionality of another major cell compartment potentially implicated in the pathogenesis of systemic JIA, the natural killer (NK) cells, were analyzed by Put *et al.* [20]. In spite of only minor alterations in phenotype and a globally intact cytotoxic profile, some defects in NK immune-regulating mechanisms were observed, including interleukin (IL)-18-induced IFNγ production and granzyme K expression. The authors speculated that the acquired impairment of NK-cell function may be part of the immune dysregulation seen in systemic JIA and constitute the link between this illness and macrophage activation syndrome (MAS).

Restriction of peripheral blood Tregulatory (Treg) cell repertoire and clonotypic expansions in both blood and synovium were found by next-generation sequencing analysis in children with JIA, but not in a sample of healthy controls and of children with Lyme arthritis. In addition, patients with JIA shared an expanded portion of synovial fluid Treg cell clonotypes that were private to JIA and not seen in Lyme arthritis. These abnormalities were thought to reflect an impairment in the competency of the Treg cell compartment and a potentially maladaptive immune response to a common antigen [21]. Rossetti *et al.* [22] identified a subset of *bona fide*, antigen-stimulated, and suppressive Treg cells that expanded during active inflammation in

JIA. These cells were found through next-generation sequencing to be enriched in synovial clonotypes, some of which were shared with pathogenic T effector (T_{eff}) cells. The selective expansion of this Treg population in active disease phases was interpreted as an attempt to counteract the growth of pathogenic T_{eff}. Another study showed that blood and synovial Tregs originate from a distinct conventional T-cell lineage and that their FOXP3 expression fluctuates in response to local environment because of interclonal and intraclonal competition [23]. Altogether, the findings of these studies delineate a highly dynamic picture, where disruption of the balance between the amount and activity of Treg populations appear regulated by complex feedback mechanisms. Thus, stabilizing FOXP3 with Treg immunomodulatory therapies could represent a potential therapeutic strategy for resolving chronic inflammation.

How environmental triggers and genetic susceptibility interact to establish and maintain the imbalance between regulatory and effector cells remains unclear. Increased attention has been focused on the potential role of the gut microbiota, and gut microbial 'pro-arthritogenic' profiles have been hypothesized. Intestinal dysbiosis has long been implicated in the pathogenesis of spondyloarthropathies. Consistently, children with enthesitis-related arthritis (ERA) exhibited increased presence of Bacteroides, a bacterial strain known for its mucine degradating properties whose arthritogenic potential has been demonstrated in animal models. [24]. Through taxon-level analysis, variations in fecal microbiota composition and reduction of microbial richness were seen among patients with ERA and polyarticular JIA, in comparison with healthy individuals. The microbiome perturbation was found to correlate with the disease status, with an increased inter-individual variability during active disease and a different pattern during remission [25]. A microbiome profile similar to that reported in type I diabetes and characterized by an abundance of Bacteroidetes and low levels of Firmicutes has been demonstrated in new-onset patients with JIA [26]. Indirect support to the pathogenic role of microbiome comes from the reported link between the risk of development of JIA and environmental factors affecting the microbiome composition, such as delivery mode and early antibiotic use [27]. Importantly, a growing body of evidence suggests that the microbiome may influence the development of the immune system, the integrity of the intestinal mucosal barrier, and the differentiation of T-cell subsets. Thus, the manipulation of the microbiome, for example by fecal microbial transplantation, may offer a perspective for future therapeutic interventions in chronic arthritis [28▪▪].

UPDATE ON TREATMENT

Although biologic agents are widely used in the management of children with polyarticular JIA, head-to-head trials comparing their efficacy and safety are lacking. In a network meta-analysis of published randomized controlled trials of abatacept (ABT), adalimumab (ADA), anakinra (ANK), etanercept (ETN), and tocilizumab (TCZ), Amarilyo *et al.* [29▪] did not found statistical differences in the efficacy and safety profile among the examined biological agents. The scrutiny of the data collected in the German BIKER registry led to conclude that ETN, ADB, and TCZ had similar efficacy in polyarticular JIA. Compliance was highest with TCZ and lowest with ADA [30].

Another German study examined the rates of serious adverse events and events of special interest under treatment with ETN and ADA in patients with polyarticular JIA observed prospectively in national registries. The frequency of serious adverse events, infections and medically important infections was significantly greater among patients treated with both ETN and ADA than in patients treated with MTX alone. However, the risk of malignancies was comparable across the three therapeutic groups. Patients receiving ETN monotherapy developed incident inflammatory bowel disease and incident uveitis more frequently than patients treated with ETN in combination with MTX or with MTX alone [31]. The possible protective role of MTX against the development of uveitis is in keeping with the hypothesis that ETN may not directly cause ocular inflammation, but the discontinuation of MTX upon successful arthritis control may pose the patient at risk [31]. Recent data have shown that MTX may prevent the onset of uveitis in children with JIA [32,33]. In a subsequent study, treatment with ETN or ADA was found to increase the rate of serious infection only slightly, compared to MTX. A higher level of disease activity, measured with the clinical JADAS10, was an independent risk factor [34▪▪].

There is increasing evidence that the individual JIA subtypes show varying responses to therapy. A systematic literature review revealed that children with extended oligoarthritis were more likely to achieve inactive disease with ETN than the other subsets, whereas no differences across subtypes were seen for ABT. Systemic arthritis was less responsive to ETN than to TCZ over 12 weeks. However, longer term response over 12 months was similar [35]. Recent evidence has been obtained that first-line

therapy with the IL-1 inhibitors ANK or canakinumab (CNK) leads to better outcomes and may potentially prevent progression to chronic polyarthritis in children with systemic JIA [36▪].

The decision about the timing of biologic treatment start should take into account the cost, which is much greater than that of MTX, and the toxicity profile. Luca *et al.* [37] compared the cost and outcomes of two strategies: MTX plus ETN as first-line therapy (ETN-first) and MTX monotherapy followed by ETN (ETN-second), using a cohort state-transition model of newly diagnosed patients with polyarticular-course JIA. They found that, over a 5-year period, first-line therapy with ETN and MTX has a relatively high cost per quality-adjusted life years compared to stepwise therapy starting with MTX alone, but may be economically advantageous for more severely affected patients.

A cooperative UK study evaluated treatment outcomes and sought for predictor of therapeutic response in 496 children with JIA over the first year of ETN administration. At 12 months, 38% of the patients reached an American College of Rheumatology Pediatric (ACR Pedi) 90 response and 48% achieved the state of minimal disease activity. Shorter disease duration, lack of corticosteroid use and history of uveitis were independent predictors of achieving an ACR Pedi 90 at 1 year, whereas a younger age and the absence of corticosteroid administration independently predicted minimal disease activity at 1 year [38].

In an Italian multicenter survey of 1038 patients with JIA treated with ETN for a median of 2.5 years, 41.8–48.6% of those still taking the medication met formal criteria for inactive disease at cross-sectional visit, 52.4% of those discontinued from etanercept had the medication stopped for clinical remission, and 55.8% of patients who were lost to follow-up were in clinical remission at last visit. ETN was overall well tolerated, as clinically significant adverse events were reported for 27.8% of patients and the medication was discontinued for side effects in 9.5% of patients. New-onset or recurrent uveitis was the most commonly reported adverse event, and there were two cases of malignancy: one thyroid carcinoma and one bladder carcinoma. One patient died for a fulminant streptococcal sepsis [39▪▪].

Thus far, most of the experience with ETN in JIA was gained in the two subsets of extended oligoarthritis and rheumatoid factor-negative polyarthritis. A recent 96-week, phase IIIb, open-label, multicenter trial (CLIPPER study) demonstrated a sustained efficacy of this medication in children with ERA and psoriatic arthritis [40].

Yokota *et al.* [41▪▪] reported the results of 1 year of post-marketing surveillance follow-up of 417 Japanese patients with systemic arthritis treated with TCZ. The proportion of patients with fever and rash decreased from baseline to 52 weeks from 54.6 to 5.6% and from 43 to 5.6%, respectively. At 52 weeks, 99% of patients had normal C-reactive protein levels. A matter of concern was that the incidence rates of both serious adverse events and serious infections were higher than those reported in previous randomized clinical trials of TCZ in systemic JIA [42,43]. The authors attributed this phenomenon to differences in patient populations, higher corticosteroid dosage, and exclusion of patients with comorbidities from clinical trials. Eight patients experienced serious infusion-related reactions, all of which occurred between the second and the fourth infusion. However, only three of these patients were discontinued from treatment. Notably, five of the six patients who were tested for anti-TCZ antibodies were positive. Twenty-four patients had 26 episodes of MAS, with no fatalities.

Instances of MAS in patients with systemic arthritis were also seen under treatment with IL-1 inhibitors [44]. Grom *et al.* [45▪] adjudicated as 'probable' or 'possible' the cases of macrophage activation syndrome recorded in clinical studies of CNK. Unexpectedly, systemic disease was well controlled in the majority of CNK-treated patients at the time of MAS. Furthermore, the difference in the rates of probable MAS between CNK-treated patients and placebo-treated patients was not significant. Infection was the most common trigger of MAS and the clinical features of the syndrome were not modified by CNK.

In the current 'biologic era', intra-articular corticosteroids (IACs) and MTX remain cornerstone medications for the therapy of JIA [46▪]. A multicenter randomized trial conducted in Italy compared IACs alone versus IACs plus MTX (administered orally) in children with oligoarticular JIA. Although in the intention-to-treat analysis of the primary outcome (remission of arthritis symptoms in all injected joints at 12 months) the difference between the two therapeutic groups was not significant, post-hoc multivariable analysis and Cox proportional hazards model suggested that concomitant administration of MTX might prolong and, to a lesser extent, augment the effectiveness of IAC therapy [47▪▪].

CONCLUSION

The pathogenetic studies of JIA published in past year have provided exciting new developments in genetic and immunologic research, which have increased our understanding of the genetic architecture and mechanisms that drive inflammation. The expansion of network analysis will help link the

immunopathogenesis to the clinical phenotypes, which should aid in the revision of classification criteria. Some important mechanisms that modulate the function of monocytes, macrophages, NK and Treg cells in the blood or synovium have been clarified, which has led to identify potential new targets for pharmacologic interventions aimed at resolving chronic inflammation. The demonstrations that the composition of the gut microbiome of children with JIA is different from that of healthy individuals suggests its involvement in disease pathogenesis and offers perspectives for future therapeutic manipulation of fecal microbial population.

Recent therapeutic studies have added significantly to the available information on the effectiveness and toxicity profile of biologic medications used in JIA. The published data have shown that around half of the patients whose disease is refractory to the conventional DMARDs, primarily MTX, may benefit significantly from the introduction of biologics. Overall, the rate of adverse events appears acceptable and no signal for an increased risk of malignancy has emerged. That the concomitant administration of MTX was found to increase the effectiveness of IAC therapy in children with oligoarticular JIA reminds that these two 'old' therapeutic interventions maintain a key role in the management of JIA.

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There are no conflicts of interest.

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Recent advances in childhood vasculitis

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Purpose of review
The review aims to summarize the recent findings in vasculitis that may have an impact in our understanding or management of these diseases.

Recent findings
We are learning more about monogenic diseases that closely mimic the pediatric vasculitides. Deficiency of adenosine deaminase 2 can present with a polyarteritis nodosa (PAN)-like picture and should be included in the differential of all pediatric cases of PAN with a family history or in cases with early stroke, or in cases resistant to conventional therapy. Mutations in tumor necrosis factor α-induced protein 3 results in a disease that can present as Behçet disease called haploinsufficiency of A20. In fact, these patients would also fulfill the existing criteria for PAN and Behçet disease, respectively. Additional advances in Behçet disease pathogenesis come from a large genetic study of Turkish Behçet disease using data obtained from genotyping using the Immunochip. This confirmed the HLA-B-51 locus as the most significant association and identified new risk loci. Large Iranian and Japanese cohorts were used as replication cohorts. Best treatment of pediatric vasculitis remains a challenge as we continue to lack controlled studies. There are new reports in treatment on Henoch–Schönlein purpura/Immunoglobulin A vasculitis which is one of our most frequent childhood vasculitides. Small series of new treatments for central nervous system vasculitis and Takayasu disease will also be summarized. Diagnostic criteria have been reassessed in pediatric Behçet disease as well as adult and childhood forms of antineutrophil cytoplasmic antibodies-positive vasculitis.

Summary
The new pathways defined in monogenic diseases may help us better understand the pathogenesis and may help us design more targeted therapy. Although pediatric cases are being increasingly recognized, the relative rarity of the diseases presents an obstacle for studies. Thus, we can reach conclusive results for their management through multicenter studies only.

Keywords
child, deficiency of adenosine deaminase 2, vasculitis

INTRODUCTION

Vasculitides are all because of inflammation of the vessel wall but they have very different presentations depending on the target vessels. It has been a never-ending exercise to try to understand the reasons for this diversity; however, progress has been rather slow except for some recent ground-breaking discoveries that advance our understanding of disease pathogenesis. The major factors hindering the progress of our understanding in vasculitides are their rarity and their complexity with regard to immune dysfunction. On the other hand, the last couple of years have taught us how defects in single genes may mimic multifactorial, more common diseases. The discoveries in the relevant areas may well lead some studies in the pathogenesis of some vasculitides as well.

A MONOGENIC DISEASE MIMICKING POLYARTERITIS NODOSA

Most of our diseases are complex, multifactorial diseases with a limited level of inheritance. We knew that systemic lupus erythematosus, a complex rather common disease, could occur in children with a rare monogenic disease such as Complement 1q deficiency. In fact this highlighted the importance of the clearing mechanisms of the complement pathways in systemic lupus erythematosus. The

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KEY POINTS

- Single gene defects may mimic complex vasculitic diseases: DADA2 may mimic PAN and haploinsufficiency of A20 may mimic Behçet disease. Thus mutations in the relevant genes should be sought for if the medical history suggests inherited disease.
- A retrospective study suggests the possible benefit of steroids in mild nephritis of HSP/IgAV.
- Adult studies are providing us with evidence for the management of rare vasculitides. Pediatricians also need to pursue multicenter studies as our adult colleagues do, otherwise our series are bound to be under powered because of small numbers.

discoveries in monogenic forms of vasculitis may inform the pathogenesis of other forms of vasculitides as well. The vasculitis world has been introduced to monogenic diseases in the past couple of years. One intriguing monogenic disease is the deficiency of adenosine deaminase 2 (DADA2) mimicking polyarteritis nodosa (PAN) [1,2]. This disease is because of mutations in the gene for cat eye syndrome chromosome region candidate 1 which encodes the protein adenosine deaminase 2 [2]. The lack of the protein is associated with endothelium damage (however, the function of this protein in the endothelial homeostasis is still unknown [3]) and is associated with a decrease of anti-inflammatory macrophages (M2). DADA2 is an autoinflammatory disease characterized by raised acute phase reactants, and features that may mimic PAN, including stroke, fever, mild immunodeficiency, fluctuating low titers of autoantibodies, and a range of other clinical symptoms. DADA2 manifested as fever, recurrent ischemic (mostly lacunar infarcts) or hemorrhagic stroke, ophthalmologic involvement (retinal artery occlusion, optic nerve atrophy, third cranial nerve palsy, diplopia because of medial rectus muscle involvement), livedo racemosa, hepatosplenomegaly, vasculitis, PAN, low serum Immunoglobulin M levels [2,3]. Currently, anti-tumor necrosis factor (TNF) therapy is the main treatment option for DADA2. Hematopoietic stem cell transplantation could be an alternative treatment for DADA2 patients [4].

One of the main manifestations of this disease is a PAN-like picture. We have recently learned more about the vasculitic spectrum of the disease through three case series: One of the single center studies analyzed 15 patients with DADA2 [5]: The clinical manifestations of adenosine deaminase 2 deficiency ranged in severity from limited cutaneous involvement to severe multisystemic vasculitis; one-third of the cases (5 of 15) were currently asymptomatic, and required close monitoring [5]. The study highlighted the importance of measuring serum adenosine deaminase 2 levels as well. Low levels of adenosine deaminase 2 enzyme activity reflects the genetic defect and is useful in establishing the diagnosis. All the six patients reported form another center either had a biopsy demonstrating necrotizing vasculitis or an angiogram showing aneurysms that had been interpreted to be typical for PAN and all met the classification criteria for childhood PAN [6]. One of the patients diagnosed as an adult, died because of necrotizing pneumonia and myelofibrosis, which has not been previously associated with DADA2. In terms of taxonomy, we suggested that DADA2 should be classified as a (secondary) vasculitis with a probable cause, because of a single gene defect and not as PAN. Both this study and that from the United Kingdom highlight the importance of considering DADA2 in PAN patients with a medical history suggestive of inherited disease or in those who are resistant to treatment [5,6].

A review on DADA2 by Caorsi *et al.* [3] also reminds us that the histopathologic features are indistinguishable from those of systemic PAN. The authors also remind us that the disease may be mild in some patients and skin limited, whereas some may present a severe, even lethal, disease with multiorgan involvement; the central nervous system involvement is rather common with ischemic or hemorrhagic strokes [3]. We have yet to learn about the clinical spectrum and pathogenesis of this monogenic disease. All three studies highlight anti-TNF as an effective treatment, whereas severe patients may require bone marrow transplantation.

BEHÇET DISEASE AND BEYOND

A monogenic form of vasculitis that mimics Behcet disease has recently been identified: haploinsufficiency of A20 which is inherited autosomal dominantly. Haploinsufficiency of A20 is caused by high-penetrance heterozygous germline mutations in TNFAIP3 (also called TNF α-induced protein 3), which encodes the nuclear factor- kappa B (NF-κB) regulatory protein A20 [7▪]. A20 restricts NF-κB signals, which subsequently leads to increased expression of inflammatory cytokines, via its deubiquitinase activity. Thus the loss of function of A20 leads to increased inflammatory products. This genetic defect results in early-onset systemic inflammation, often mimicking Behçet disease [7▪]. The six unrelated families reported by Zhou *et al.* had features meeting the criteria for Behçet disease with ulcers, eye disease, and gastrointestinal manifestations [7▪].

A nice study in Behçet disease has provided some important insights into the pathogenesis of Behçet disease this year: An immunochip was designed to further clarify the genetic cause of Behçet disease in the previously studied Turkish cohort and some of the results were replicated in Japanese and Iranian Behçet disease patients [8▪]. This nice study by Takeuchi *et al.* has provided important insights into the pathogenesis of Behçet disease [8▪]. They used the commercially available Immunochip to further clarify the genetic cause of Behçet disease by analyzing 1900 Turkish Behçet's disease cases and 1779 controls. This study again identified HLA-B∗51 as the strongest association. And they identified new risk loci including Interleukin 1A–Interleukin 1B, interferon regulatory factor 8, and CCAAT/Enhancer Binding Protein Beta–Protein Tyrosine Phosphatase 1, in the Turkish cohort. They were able to replicate these new risk loci in either an Iranian or Japanese cohorts. The Interleukin 1 region showed a significant association in the Turkish group only, which is associated with increased Interleukin 1 β production. It was interesting that other loci such as Laccase Domain Containing 1 and fucosyltransferase 2 that are associated with microbial responses were replicated in all three groups, (these loci are shared with Crohn's disease as well) [8▪]. These results implicate innate immune response to microbes in Behçet disease susceptibility. Thus, we may start to understand why Behçet disease was confined to the Silk Road.

Another important study in Behçet disease has been the new classification/diagnostic criteria for children with Behçet disease [9]. After a prospective 8-years work and a large registry of 219 patients from 42 centers, an international group of pediatricians have suggested the requirements to classify a child as Behçet disease as the presence of at least three of the following criteria: recurrent oral aphtosis (at least three attacks per year), genital ulcers, skin features, eye disease, neurological features, and vasculitic features. Skin features are specified as necrotic folliculitis, acneiform lesions, or erythema nodosum and vasculitic features are venous thrombosis, arterial thrombosis, and arterial aneurysm [9].

IMMUNOGLOBULIN A VACULITIS/HENOCH–SCHONLEIN PURPURA

Immunoglobulin A Vasculitis (IgAV) or Henoch–Schonlein purpura (HSP) is still one of the most common vasculitis of childhood. It is ironic that although it is so common we still lack high evidence data for the management of these patients. Two studies have analyzed outcome in their IgAV HSP patients.

A study attempted to define the effect of treatment on outcome in Class 2 nephritis of HSP/IgAV which describes mesangial nephritis without crescents [10]. Between 1995 and 2015, 92 children with class 2 HSP nephritis were collected retrospectively with a median follow-up of 36 months [10]. At the end of follow-up although no patient had a GFR less than 60, 31% had less than 90 ml/min/1.73 m^2. In total, 25% had persistent proteinuria at the end of the follow-up. Patients treated with steroids had a higher probability of full remission (87 versus 68%) although the difference was not significant. The authors have commented that the better outcome in patients treated with steroids favored their use in kidney involvement of this stage [10]. This also reflects our common practice however, prospective controlled studies are clearly needed to decide on the duration and dose of treatment.

A single center study of 417 patients attempted to analyze the predictors of relapses [11]. In total, 32% of the patients had at least one relapse. The median time interval to relapse was 1 month after diagnosis and the median number of relapses in this group was one. Joint and gastrointestinal manifestations had a higher likelihood to relapse whereas an infectious trigger was associated with less relapse [11].

Pediatricians have often come across the question of whether vaccines have triggered vasculitis. The Brighton collaboration vasculitis working group has done a systematic literature review to analyze evidence and current reporting practice of vasculitides including HSP/IgAV [12]. Although there were a number of case reports suggesting possible relation, the authors concluded that the larger studies with higher quality studies failed to show a causal association between vaccination and subsequent development of vasculitis, including HSP/IgAV [12]. However, they suggest that a standardized collection, analysis, and uniform definition of HSP/IgAV is needed to improve data interpretation for final conclusions.

RARE VASCULITIDES

Childhood primary angiitis of central nervous system (PACNS) remains as a challenging topic as the treatments are not based on controlled trials. Mycophenolate mofetil was shown to be safe and beneficial in four PACNS patients as an induction and maintenance therapy (750–1000 mg/m^2, half-dose for the first 10–15 days followed by full-dose). Adding mycophenolate mofetil to anticoagulants and glucocorticoid treatment resulted in the induction and maintenance of clinical remission (median treatment 29 months) [13].

Imaging methods are essential for PACNS diagnosis as it is one of the three criteria still used for both adult and childhood PACNS diagnosis [14]. These criteria are: a newly acquired neurological deficit (stroke, seizures, movement disorder, optic neuritis, progressive cognitive decline, headaches, or psychiatric manifestations including behavior changes); angiographic and/or histologic evidence of central nervous system vasculitis and the absence of systemic condition associated with these findings. For understanding vascular abnormalities, magnetic resonance angiography (MRA) is the initial method but particularly, for MRA normal cases or for revealing posterior circulation vascular pathology, conventional angiography should be considered. Two recent studies emphasized the importance of vascular imaging techniques. MRA images reconstructed with maximum intensity projection showed progressive paucity of peripheral vessels proposed as a reflection of the inflammation of the peripheral vasculature [15]. This reconstruction and higher resolution methods improve the sensitivity of MRA. Elbers *et al.* [16] presented their 12-month follow-up study with MRA: they highlight that discordant vascular progression (described as a new or worsening arterial abnormality coexisting with an improved or normalized vessel) was significantly associated with stroke recurrence. This discordance thus seems to be another important feature that we should follow in our patients.

One of the vasculitides that we lack high evidence for management is Takayasu arteritis (TA). There was an excellent randomized double-blind trial for abatacept from the adult rheumatologists for TA: 34 patients were randomized to receive either prednisone and abatacept or prednisone and placebo [17]. The primary endpoint was relapse-free survival. Unfortunately, the relapse-free survival was not significantly different at 12 months and the median duration of remission was the same in both groups [17].

A small single center study has addressed the use of anti-Interleukin 6 in four children with TA: three were resistant to conventional disease-modifying anti-rheumatic drugs and the fourth one had refused cyclophosphamide treatment [18]. All four patients achieved remission at 3 months suggesting that tocilizumab may be an effective alternative in the treatment of these patients [18] However, the adult data warns against the risk of relapses after cessation of treatment and thus long follow-up studies are needed for proper recommendations.

And finally, a study from our colleagues in adult rheumatology addresses a discussion that has been going on in the recent years, whether ANCA-associated vasculitis should be classified according to the clinical features or the specificity antineutrophil cytoplasmic antibodies (ANCA) as proteinase 3 or myeloperoxidase related disease. Cornec *et al.* [19] reviewed the evidence for ANCA specificity to define a more homogeneous group of disease versus using the clinical diagnosis of microscopic polyangiitis (MPA) and granulomatosis with polyangiitis (GPA). The authors review the difference in animal models (that does not exist for PR3), the genetic associations with ANCA specificity and the association with the course of the disease. They thus conclude that ANCA specificity should be used rather than the clinical diagnosis [19]. However, the jury is still out to reach final conclusions on where to place eosinophilic GPA and granulomatous lesions in such a classification. On the other hand, in a pediatric series of 48 children the features of MPA and GPA (Wegener granulomatosis) were compared and the differences were highlighted [20]. The authors found that a younger age of onset and, gastrointestinal manifestations and severe kidney disease are more likely seen in children with MPA compared to those with GPA.

CONCLUSION

The review has addressed some of the recent advances in childhood vasculitis in the last year. The pediatric community should start multicenter collaborations as in juvenile idiopathic arthritis regarding management and the treatment of vasculitides. The author apologizes for important studies that may have not been covered.

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Conflicts of interest

There are no conflicts of interest.

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Osteoporosis in childhood

Francesco Vierucci[a], *Giuseppe Saggese*[b], *and Rolando Cimaz*[c]

Purpose of review
The aim of this review is to highlight recent findings in prevention, diagnosis, and treatment of pediatric osteoporosis.

Recent findings
Several genes are involved in bone mass acquisition, and various monogenic bone disorders characterized by reduced bone mineral density and increased bone fragility have been recently described. Moreover, many chronic diseases and/or their treatment have been associated with impaired bone mass acquisition. Pediatric osteoporosis should be adequately suspected and properly diagnosed in children at risk of fractures. Particularly, detection of vertebral fracture allows the diagnosis regardless of densitometric evaluation. Dual X-ray absorptiometry remains the most widely used densitometric technique in childhood, but interpretation of results should be made with caution because of different confounding factors. Bisphosphonates represent one of the main medical treatments of pediatric osteoporosis, and many different protocols have been proposed. Bisphosphonates administration should be characterized by a first phase, followed by a period of maintenance. Optimal route of administration, duration of therapy, and long-term safety of bisphosphonates treatment require further investigation.

Summary
Careful monitoring of children at risk of fractures is essential to pose early diagnosis of osteoporosis. In children with persistent risk factors and reduced probability of spontaneous recovery, medical treatment with bisphosphonates should be considered.

Keywords
bisphosphonates, bone mineral density, childhood, fractures, osteoporosis

INTRODUCTION

Adult osteoporosis may be the consequence of impaired bone mass acquisition during pediatric age. In recent years, increasing attention has been paid to the bone health of children and adolescents in order to optimize bone mass accrual and avoid detrimental effects later in life, such as the occurrence of osteoporotic fractures. This is particularly true for children with genetic conditions associated with bone fragility or those affected by chronic diseases that negatively influence bone mass [1]. The present review will focus on recent advances in prevention, diagnosis, and management of pediatric osteoporosis.

ACQUISITION OF BONE MASS DURING PEDIATRIC AGE

Bone mass acquisition during childhood culminates in the achievement of peak bone mass (PBM), the amount of bone mass acquired when accrual plateaus after completion of growth and development [2▪▪]. The timing of PBM differs depending on the skeletal site considered, sex, maturational timing, and lifestyle factors. Bone status during pediatric age is a strong predictor of bone status in young adulthood when PBM is achieved, as bone mass tracks during childhood and adolescence [3]. Even if up to 80% of bone mass acquisition depends upon genetic factors [4], environmental factors such as calcium intake, vitamin D status, and physical activity play a pivotal role [2▪▪,5]. The Bone Mineral Density in Childhood study longitudinally assessed calcium intake in 1743 children, showing that calcium intake had a significant effect on bone accrual at lumbar spine in non-black girls [6]. In healthy Flemish children, physical

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KEY POINTS

- Primary prevention of osteoporosis starts during childhood and adolescence, by ensuring a balanced diet with sufficient calcium intake, sufficient vitamin D status, and regular physical exercise.
- The term osteogenesis imperfecta, the most prevalent form of primary osteoporosis in children, identifies a wide spectrum of genetic conditions ranging from mild forms to perinatally lethal ones.
- Glucocorticoid-associated osteoporosis is a frequent complication of childhood systemic inflammatory diseases and the most common form of secondary osteoporosis.
- Pediatric osteoporosis can be diagnosed in presence of at least one vertebral compression fracture not related to local disease or high-energy trauma (regardless of densitometry measurements), or in presence of both reduced bone mass and a clinically significant fracture history.
- Medical treatment with bisphosphonates should be considered in children with osteoporosis, persistent risk factors, and reduced probability of spontaneous recovery.

activity and diary consumption were positively related to whole body bone mass, with a negative effect of sedentary behavior [7]. A systematic review of randomized controlled trials (RCTs) also showed that adequate calcium intake and regular physical activity sinergically improve bone health in children and adolescents [8]. Body composition significantly influences bone health during childhood, and lean mass was demonstrated to have a positive effect on bone mass and a larger contribution to the variance of bone parameters than fat mass [9▪]. Whether obesity represents a risk factor for childhood bone status is still debated [10], but a Mendelian randomization study showed that adiposity is causally related to increased bone mineral density (BMD) at all sites except the skull, possibly reflecting positive effects of loading on bone accrual at weight bearing sites [11▪]. A recent meta-analysis evaluated bone mass in patients with eating disorders, confirming an association between low BMD and conditions characterized by nutritional deprivation and altered body composition [12▪]. Vitamin D directly influences bone mass accrual contributing to the regulation of calcium–phosphorus metabolism, and indirectly stimulating the development of muscle tissue [13–15]. Duodenal expression of 25-hydroxyvitamin D3–1α-hydroxylase is higher in adolescents than in children and adults, representing a metabolic adaptation that promotes dietary calcium absorption for the growing bone [16]. Serum 25-hydroxyvitamin D [25(OH)D] levels correlated with some important bone density and bone quality parameters in adolescents [17▪]. At present, the cut-off to define vitamin D sufficiency is still debated, particularly in childhood. A recent global consensus focused on nutritional rickets prevention defined vitamin D sufficiency for 25(OH)D levels more than 20 ng/ml. However, several other consensus and guidelines that considered also pleiotropic effect of vitamin D suggested 30 ng/ml as cut-off for sufficiency [18,19▪▪].

The National Osteoporosis Foundation applied an evidence based grading system to describe the strength of available evidence on modifiable lifestyle factors that may influence the acquisition of PBM. This review reported strong evidence (grade A) for calcium and moderate evidence (grade B) for dairy products and vitamin D. Physical activity and exercise were also found to determine important effects on bone mass and density (grade A) and on bone structural outcomes (grade B) [20▪▪]. Four out of eight included RCTs provide evidence for a beneficial effect of vitamin D supplementation on bone accrual, mainly in subjects with vitamin D deficiency. At present, several unanswered questions remain (critical times during which supplementation may be most effective, continuous or intermittent supplementation, sex difference), thus vitamin D supplementation to optimize bone mass acquisition should be reserved for children at risk for deficiency.

CAUSES OF OSTEOPOROSIS IN CHILDHOOD

Pediatric osteoporosis is usually divided in primary and secondary forms, as reported in recent reviews [1,21,22▪▪,23,24]. Various monogenic bone disorders with reduced BMD and increased bone fragility have been described [25–27,28▪]. Osteogenesis imperfecta is the most prevalent form of primary osteoporosis in children, even if the exact incidence is still unknown. The term osteogenesis imperfecta identifies a wide spectrum of conditions ranging from mild forms to perinatally lethal ones. Although for three decades it has been recognized that the majority of patients with osteogenesis imperfecta had mutations in *COL1A1* and *COL1A2* genes, defects in several other genes have been recently demonstrated to determine osteogenesis imperfecta. At present, Online Mendelian Inheritance of Man (OMIM) database has identified 17 genotypic osteogenesis imperfecta types, and it seems plausible that new osteogenesis imperfecta-associated genes may be identified in the near future. To simplify this complex scenario, in 2010 the revision of nosology and classification of genetic

skeletal disorders suggested to update the original Sillence classification defining five clinical osteogenesis imperfecta types (Table 1) [29,30]. Moreover, other genetic conditions are associated with osteoporosis and bone fragility, clinically overlapping osteogenesis imperfecta (Table 2). Fracture history, clinical examination, bone and mineral biochemistry, X-rays (lumbar and long bones), and densitometric assessment allow a clinical diagnosis of osteogenesis imperfecta that should be confirmed with molecular analysis [31▪].

Secondary forms of pediatric osteoporosis are caused by detrimental effects of systemic diseases and/or their treatment on bone mass accrual (Table 3). The impact of specific conditions on bone health has been extensively reviewed [21,32–34]. During the course of chronic disorders several factors may interact to determine osteoporosis other than direct bone detrimental effects of the disease or its treatment, such as prolonged immobilization, reduced time spent outdoor and possibly consequent vitamin D deficiency, hypogonadism, and poor nutrition. Inflammatory systemic diseases are characterized by increased levels of proinflammatory cytokines (such as tumor necrosis factor alpha, interleukin-1, and interleukin-6) that uncouple bone remodeling cycle, interfering with bone mass acquisition [24]. Childhood rheumatic diseases are associated with reduced BMD and increased risk of vertebral and nonvertebral fractures. This association is robust for juvenile idiopathic arthritis, whereas studies on juvenile systemic lupus erythematosus or juvenile dermatomyositis are more limited [35▪]. Glucocorticoid-associated osteoporosis is a frequent complication of childhood systemic inflammatory diseases and the most common form of secondary osteoporosis. Glucocorticoids are physiological required for normal bone development because of their regulation of osteoblast differentiation, probably by Wnt/β-catenin pathway and TSC22D3 [36]. On the contrary, glucocorticoid treatment directly alters bone remodeling increasing bone resorption and decreasing bone formation, and indirectly affecting muscle tissue. Finally, glucocorticoids affect calcium homeostasis by increasing its urinary excretion and reducing gastrointestinal absorption [37]. Inhaled corticosteroids may also impact skeletal growth and bone accrual [38▪], particularly during the first 1–2 years of treatment [39] and in children exposed before 6 years of age [40].

DIAGNOSIS OF OSTEOPOROSIS IN CHILDHOOD

In 2013, the International Society for Clinical Densitometry (ISCD) recommended that pediatric osteoporosis can be diagnosed in presence of at least one vertebral compression fracture not related to local disease or high energy trauma (regardless of densitometry measurements), or in presence of both reduced bone mass [bone mineral content (BMC) or BMD $\leq$2 *Z*-score, taking account for bone dimensions] and a clinically significant fracture history ($\geq$2 long bone fractures before 10 years of age or $\geq$3 long bone fractures during the 10–19 years period) [41,42]. To avoid unnecessary investigations, fracture history assessed by questionnaire should be confirmed evaluating medical documentation [43▪]. Dual-energy X-ray absorptiometry (DXA) is the preferred method to asses bone mass during pediatric age because of good reproducibility and speed, reduced exposure to ionizing radiation, and large availability of reference data [44]. Peripheral quantitative computed tomography (pQCT) separately analyzes trabecular and cortical bone compartments, allowing the analysis of appendicular bone geometry, density, and strength, and to evaluate fat and muscle composition of the limbs. However, pQCT use is still limited by the lack of standardized scanning protocols and normative pediatric values [45,46].

In 2016, the American Academy of Pediatrics (AAP) updated 2013 ISCD report regarding the role of bone densitometry in children [47▪]. Both ISCD and AAP recommended to perform DXA evaluation in children with bone fragility at lumbar spine (L1–L4) and total body less head. Indeed, skull mineralization is not affected by nutritional or environmental factors such as physical exercise, and skull fractures should not suggest osteoporosis [48▪]. Lateral distal femur scan may be performed in children with spinal deformity or contractures [49,50]. Even if DXA represents a valuable tool as part of a comprehensive skeletal assessment, at present the diagnosis of pediatric osteoporosis cannot be established on the basis of densitometry criteria alone [47▪]. The term osteopenia or osteoporosis should not appear in pediatric DXA reports, whereas the detection of BMC or areal BMD Z-score $\leq$ 2.0 SD should be identified with the term 'low bone mass or BMD' [44]. DXA interpretation may be difficult, particularly in short patients with smaller bones. The difficulty in accounting for reduced bone size increases in children with chronic diseases as they usually present delayed growth and/or pubertal development. Several body size adjustment techniques have been developed, but at present none adequately addresses all the potential concerns nor has been validated in terms of incident fracture prediction [48▪].

The assessment of lateral spine images acquired by DXA may be used to detect vertebral fracture, a technique named vertebral fracture assessment

Table 1. Classification of types of osteogenesis imperfecta as reported by OMIM (https://www.omim.org/) and by 2010 revision of nosology and classification of genetic skeletal disorders [29]

MIM osteogenesis imperfecta type	Phenotype MIM number	Inheritance	Gene	Gene MIM number	Chromosomal location	Protein product	Disease type	Skeletal manifestations
I	#166200	AD	*COL1A1*	120150	17q21.33	Collagen type I alpha 1 chain	Type 1: non deforming with blue sclerae	Skull: wormian bones Spine: biconcave flattened vertebrae Limbs: occasional femoral bowing, mild joint hypermobility
II	#166210 #166210	AD AD	*COL1A1* *COL1A2*	120150 120160	17q21.33 7q21.3	Collagen type I alpha 1 chain Collagen type I alpha 2 chain	Type 2: perinatally lethal	Skull: wormian bones, soft calvarial bones, large fontanels Spine: platyspondyly Pelvis: hips usually flexed and abducted (frog-leg position), flattened acetabulae and iliac wings Limbs: bowing of long bones
III	#166210 #166210	AD AD	*COL1A1* *COL1A2*	120150 120160	17q21.33 7q21.3	Collagen type I alpha 1 chain Collagen type I alpha 2 chain	Type 3: progressively deforming	Skull: wormian bones, large fontanels, soft calvarial bones Spine: scoliosis, kyphosis, codfish vertebrae Pelvis: protrusio acetabuli Limbs: thin gracile long bones, tibial bowing, short femurs, 'popcorn' calcification
IV	#166210 #166210	AD AD	*COL1A1* *COL1A2*	120150 120160	17q21.33 7q21.3	Collagen type I alpha 1 chain Collagen type I alpha 2 chain	Type 4: moderate severity with normal sclerae	Skull: wormian bones Spine: scoliosis, kyphosis, biconcave flattened vertebrae Limbs: femoral bowing present at birth, straightening with time; bowed limbs
V	#610967	AD	*IFITM5*	610967	11p15.5	Interferon-induced transmembrane protein 5	Type 5: calcification in interosseuous membranes	Skull: wormian bones Spine: biconcave, wedge-shaped, and flattened vertebrae Pelvis: irregular, meshlike matrix lamellae in the histology of the iliac crest Limbs: limited pronation/supination of forearm, anterior dislocation of radial head, calcified interosseuous membrane (forearms), hyperplastic callus, metaphyseal bands adjacent to growth plate, hyperextensible joints
VI	#613982	AR	*SERPINF1*	172860	17p13.3	Serpin peptidase inhibitor, clade F, member 1	Type 3: progressively deforming	'Fish-scale' pattern of the lamellae and presence of excessive osteoid at histology of iliac biopsy specimens

Table 1 (*Continued*)

MIM osteogenesis imperfecta type	Phenotype MIM number	Inheritance	Gene	Gene MIM number	Chromosomal location	Protein product	Disease type	Skeletal manifestations
VII	#610682	AR	*CRTAP*	605497	3p22.3	Cartilage-associated protein	Type 2: perinatally lethal Type 3: progressively deforming Type 4: moderate severity with normal sclerae	Skull: wormian bones Spine: scoliosis Pelvis: coxa vara, protrusio acetabuli Limbs: rhizomelia, micromelia, bowing and shortening of long bones
VIII	#610915	AR	*P3H1 (LEPRE1)*	610339	1p34.2	Prolyl 3-hydroxylase 1	Type 2: perinatally lethal Type 3: progressively deforming	Skull: poorly ossified skull, wormian bones Spine: platyspondyly, scoliosis, kyphosis Limbs: thin-gracile long bones, bowing of long bones, bulbous metaphyses
IX	#259440	AR	*PPIB*	123841	15q22.31	Cyclophilin B	Type 2: perinatally lethal Type 3: progressively deforming Type 4: moderate severity with normal sclerae	Spine: scoliosis, kyphosis Limbs: bowing of long bones
X	#613848	AR	*SERPINH1*	600943	11q13.5	Serpin peptidase inhibitor, clade H, member 1	Type 3: progressively deforming	Spine: platyspondyly, scoliosis Limbs: bowing and shortening of long bones, genu valgum, generalized joint laxity
XI	#610968	AR	*FKBP10*	607063	17q21.2	FK506-binding protein 10	Type 3: progressively deforming	Skull: wormian bones Spine: wedge-shaped and biconcave vertebrae, scoliosis, kyphoscoliosis Pelvis: coxa vara, protrusio acetabuli, 'fish-scale' pattern of lamellae, increased osteoid volume Limbs: bulbous metaphyses, bowing of long bones
XII	#613849	AR	*SP7*	606633	12q13.13	Specificity protein 7	Type 4: moderate severity with normal sclerae	Skull: wormian bones Spine: mild scoliosis Limbs: bowing of long bones

Table 1 (*Continued*)

MIM osteogenesis imperfecta type	Phenotype MIM number	Inheritance	Gene	Gene MIM number	Chromosomal location	Protein product	Disease type	Skeletal manifestations
XIII	#614856	AR	*BMP1*	112264	8p21.3	Bone morphogenetic protein 1	Type 3: progressively deforming	Skull: wormian bones Spine: kyphoscoliosis, platyspondyly Limbs: bowing of long bones, limited movements of the knee joints, lack of bone modelling with wide distal metaphyses of femora, serpentine thin tibiae and fibulae
XIV	#615066	AR	*TMEM38B*	611236	9q31.2	Transmembrane protein 38B	Type 3: progressively deforming	(Described in 3 consanguineous Saudi families with a history of fractures)
XV	#615220	AR	*WNT1*	164820	12q13.12	Wingless-type MMTV integration site family, member 1	Type 3: progressively deforming	Skull: soft calvarial bones Spine: scoliosis, platyspondyly Limbs: bowing and shortening of long bones, hypermobility of joints, marked bilateral angulation of the proximal femur
XVI	#616229	AR	Deletion in *CREB3L1*	616215	11p11.2	Old astrocyte specifically induced substance	Type 2: perinatally lethal	Skull: soft calvarial bones, large fontanels Limbs: accordion-like broadened appearance of tubular bones, bowing of long bones
XVII	#616507	AR	*SPARC*	182120	5q33.1	Osteonectin	Type 4: moderate severity with normal sclerae	Spine: platyspondyly, scoliosis Limbs: joint hyperlaxity

AD, autosomal dominant; AR, autosomal recessive.

Table 2. Other causes of primary osteoporosis with clinical features overlapping osteogenesis imperfecta

Disease	Phenotype MIM number	Inheritance	Gene	Gene MIM number	Chromosomal location	Protein product	Skeletal manifestations
Bruck syndrome type 1	#259450	AR	*FKBP10*	607063	17q21.2	FK506-binding protein 10	Congenital joint contractures (knee, ankle, hip, elbow), joint laxity (fingers and wrist) Spine: kyphosis, scoliosis, flattened vertebral bodies, vertebral wedging Pelvis: protrusio acetabuli, coxa vara
Bruck syndrome type 2	#609220	AR	*PLOD2*	601865	3q24	Lysyl hydroxylase 2	Congenital joint contracture Skull: wormian bones Spine: platyspondyly Limbs: femoral bowing
Osteoporosis-pseudoglioma syndrome	#259770	AR	*LRP5*	603506	11q13.2	Low-density lipoprotein receptor-related protein 5	Skull: intraocular calcification Spine: kyphoscoliosis, platyspondyly Limbs: joint laxity, narrow diaphyses, wide metaphyses, long bone deformities
Familial doughnut lesions of skull	#126550	AD	–	–	–	–	Skull: hyperostotic or osteosclerotic lesions
Idiopathic juvenile osteoporosis	259750	–	[a]	–	–	–	Bone fragility
Cole-Carpenter syndrome 1	#112240	AD	*P4HB*	176790	17q25.3	Procollagen-proline, 2-oxoglutarate-4-dioxygenase, beta subunit	Skull: soft calvarial bones, frontal and coronal craniosynostosis Spine: scoliosis Pelvis: decreased trabecular volume and bone formation on transiliac biopsy Limbs: marked deformity of long bones, 'popcorn epiphyses' in the distal femora and proximal tibiae
Cole-Carpenter syndrome 2	#616294	AR	*SEC24D*	607186	4q26	SEC24-related gene family, member D	Skull: turricephaly at birth, macrocephaly, skull erosions, skull ossification defect, craniosynostosis, wormian bones Spine: flattened vertebrae, thoracic kyphosis Pelvis: hypoplastic acetabular roof, high and narrow iliac wings Limbs: deformities of long bones
Gnathodiaphyseal dysplasia	#166260	AD	*ANO5*	608662	11p14.3	Anoctamin 5	Skull: cemento-osseous lesions (maxilla and mandible), Jaw lesions show fibroblasts in fibrous stromal tissue Limbs: bowing of long bones, diaphyseal cortical sclerosis
Geroderma osteodysplasticum	#231070	AR	*GORAB*	607983	1q24.2	Rab6-interacting golgin	Spine: progressive kyphoscoliosis, vertebral collapse Limbs: bowing of long bones
X linked osteoporosis	#300910	XL	*PLS3*	300131	Xq23	Plastin 3	Spine: vertebral compression fractures Pelvis: pelvic fractures Limbs: hypermobility of elbow and knee joints

Table 2 (*Continued*)

Disease	Phenotype MIM number	Inheritance	Gene	Gene MIM number	Chromosomal location	Protein product	Skeletal manifestations
Hajdu-Cheney syndrome	#102500	AD	*NOTCH2*	600275	1p12	NOTCH, drosophila, homolog of, 2	Skull: bathrocephaly, wormian bones, small mandible Spine: narrow disc space, biconcave vertebrae, kyphoscoliosis, cervical instability, vertebral collapse Limbs: joint laxity, genu valgum, dislocation of radial head
Spondylo-ocular syndrome	#605822	AR	*XYLT2*	608125	17q21.33	Xylosyltransferase 2	Skull: soft calvarial bones Spine: generalized vertebral flattening, vertebral compression fractures Limbs: deformities of long bones
Infantile hypophosphatasia	#241500	AR	*ALPL*	171760	1p36.12	Alkaline phosphatase	Skull: soft calvarial bones, large fontanels, craniosynostosis Spine: vertebral clefts, platyspondyly Limbs: micromelia, bowing and shortening of long bones, 'spurs' in midshaft of ulna and fibula, metaphyseal cupping

AD, autosomal dominant; AR, autosomal recessive; XL, X-linked.
[a]Heterozygous, inactivating mutations causing juvenile osteoporosis have been found in *WNT1* and *LRP5* genes.

(VFA). VFA was confirmed to be a practical screening tool for identification of vertebral fracture in children at risk of fragility fractures [51▪]. Despite the advantage that VFA exposes the patient to low radiation and can be performed during a routine DXA evaluation, VFA assessment of vertebral bodies in the mid-thoracic region is still suboptimal, particularly in younger children [52].

Recently, vertebral fracture incidence rates of 4.4 per 100 person-years among 134 children with rheumatic disorders [53] and of 8.7 per 100 person-years among 186 children with acute lymphoblastic leukemia have been reported [54]. One-third to one-half of fractured patients were asymptomatic. Thus, children at risk should be routinely assessed for vertebral fracture, even in the absence of back pain (see section 'Prevention and Treatment of Osteoporosis in Childhood'). Moreover, early diagnosis of vertebral fracture is important also because mild (grade 1) vertebral fracture independently predict future fractures [54].

A detailed algorithm for diagnosis and treatment of pediatric osteoporosis has been proposed by Ward *et al.* In children with suspected bone fragility rickets should be first excluded, and if present calcium, phosphate, and vitamin D deficiencies should be treated. In the absence of underlying systemic conditions that may cause secondary osteoporosis, primary forms may be suspected and eventually confirmed with molecular analysis, starting with *COL1A1* and *COL1A2* [22▪▪].

PREVENTION AND TREATMENT OF OSTEOPOROSIS IN CHILDHOOD

Primary prevention of osteoporosis starts during childhood, ensuring a balanced diet with sufficient calcium intake, a sufficient vitamin D status, and regular physical exercise (possibly outdoor to promote skin synthesis of vitamin D). This approach is particularly important in children with chronic conditions that negatively affect bone accrual. For example, children treated with glucocorticoids or anticonvulsant medication should receive at least twice the amount of vitamin D than the dose recommended for age [55]. Vitamin D supplementation up to 2000 IU/day has been suggested in children with rheumatic disorders treated with glucocorticoids [56]. Adequate treatment of the underlying illness is obviously also essential to prevent and treat osteoporosis; in particular, it is important to use the lowest possible glucocorticoid dose that maintains disease control [22▪▪].

Secondary prevention strategies are reserved for children at high risk to identify early signs of osteoporosis. A baseline spine X-ray or VFA assessment

Table 3. Main causes of secondary pediatric osteoporosis

Chronic diseases
Malignancy (leukemia, lymphoma)
Rheumatologic disorders (juvenile idiopathic arthritis, systemic lupus erythematosus, juvenile dermatomyositis, etc.)
Cystic fibrosis
Inflammatory bowel disease
Renal disease
Transplantation
Hepato-biliary diseases (cholestatic forms)
Cyanotic congenital heart disease
Thalassemia
Malabsorption syndromes, celiac disease
Epidermolysis bullosa
Neuromuscular disorders
Cerebral palsy
Rett syndrome
Duchenne muscular dystrophy, other myopathic diseases
Spina bifida
Spinal muscular atrophy
Other diseases associated with chronic immobilization
Endocrine disorders
Cushing syndrome
Growth hormone deficiency
Hyperthyroidism
Hypogonadism, anorexia nervosa, female athletes
Panhypopituitarism
Type 1 diabetes
Genetic diseases
Turner syndrome
Klinefelter syndrome
Lysinuric protein intolerance
Glycogen storage disease
Galactosemia
Gaucher disease
Iatrogenic
Glucocorticoids
Methotrexate
Cyclosporine
Heparin
Radiotherapy
GnRH agonist
Medroxyprogesterone acetate (long-term use)
Anticonvulsants (phenytoin, phenobarbital, carbamazepine)

should be offered to children treated with glucocorticoids for at least 3 months, with a follow-up at 12 months and subsequently every 12–24 months if treatment with glucocorticoids continues. Children with neuromuscular disorders and impaired mobility should also receive regular spine X-ray evaluations starting from 6 to 8 years until growth completion [22▪▪]. In children at high risk for fractures, a DXA evaluation should be performed at least as frequently as radiography, with a minimum interval of 6–12 months [22▪▪,41]. As BMD tracks during childhood, repeated DXA scans may be useful to identify children with a significant probability of spontaneous recovery or those necessitating treatment [24].

At present, the antiresorptive agents bisphosphonates represents the main medical treatment of pediatric osteoporosis, and many different protocols have been proposed. Mechanism of action, pharmacokinetics, dose, and possibly adverse effect of bisphosphonates have been recently reviewed [57,58]. As data on long-term efficacy and safety of bisphosphonates are lacking, the issue regarding when to start such treatment is still debated. In their algorithm, Ward *et al.* suggested to initiate bisphosphonates administration in subjects with diagnosed primary or secondary osteoporosis and low-trauma long bone or vertebral fractures. The impact of the fractures on quality of life and lack of potential for spontaneous recovery because of persistent osteoporosis risk factors should also be considered [22▪▪]. Some recent systematic reviews and meta-analyses evaluated the effect of bisphosphonates administration in specific forms of pediatric osteoporosis (Table 4). Current evidence suggests a positive effect of bisphosphonates in increasing BMD in children with osteogenesis imperfecta [59,60–62▪▪], but significant effect of bisphosphonates in reducing fracture risk and improving quality of life is still debated. Limited evidence also suggests a positive effect of bisphosphonates in children with cerebral palsy [64,65▪▪] or glucocorticoid-induced low BMD [66]. On the contrary, there is inconclusive evidence to recommend bisphosphonates administration in children with acute lymphoblastic leukemia to alter osteonecrosis disease progression [63▪], or with anorexia nervosa [67].

Current recommendations suggest that bisphosphonates administration should be characterized first by a stabilization phase, usually lasting at least 2 years, followed by a maintenance one. Once clinical stability has been obtained (defined as absence of new vertebral fracture in previously normal vertebral bodies, absence of additional loss of vertebral height at sites of previous fractures, eventual reshaping of vertebral fracture, absence of new nonvertebral fractures, bone and back pain, improvement in mobility and in lumbar BMD), a lower dose (half-dose or less) should be administered in presence of persistent risk factor for osteoporosis

Table 4. Systematic reviews and/or meta-analyses regarding bisphosphonates administration in children with osteoporosis published from 2015

First author	Disease	Studies included	Population	*N* of patients receiving bisphosphonates	Conclusions
Rijks [59]	Osteogenesis imperfecta	10	Children	519	Treatment with oral or intravenous bisphosphonates resulted in an increase in BMD and seems to be safe and well tolerated in children with osteogenesis imperfecta.
Sinikumpu [60]	Osteogenesis imperfecta type III[a]	10	Children	346	Bisphosphonates leaded to better life conditions in these patients. Particularly, bisphosphonates have revolutionized the treatment of newborns with severe osteogenesis imperfecta type III.
Shi [61]	Osteogenesis imperfecta	9	Children and adults	557	Bisphosphonates could increase BMD and reduce the risk of facture in patients with osteogenesis imperfecta. There was no enough evidence to identify differences in efficacy between oral and intravenous bisphosphonates on fracture reduction.
Dwan [62▪▪]	Osteogenesis imperfecta	14	Children and adults	819	Current evidence, albeit limited, demonstrates that oral or intravenous bisphosphonates increase BMD in children and adults with osteogenesis imperfecta. Studies included do not show if bisphosphonates conclusively improve clinical status (pain, growth, and functional mobility).
Amin [63▪]	Osteonecrosis in ALL	5	Children	64	There is currently no evidence that bisphosphonates alter osteonecrosis disease progression in childhood ALL.
Kim [64]	Cerebral palsy	4	Children	64	Bisphosphonates have a significant effect on improving BMD in children with cerebral palsy. Further standardization of treatment protocols including treatment dosage and duration needs to be established.
Ozel [65▪▪]	Cerebral palsy[a]	5	Children	[b]	Bisphosphonates are probably effective in increasing BMD in children with cerebral palsy.
Jayasena [66]	Glucocorticoid-induced low BMD[a]	4	Children	43	For children who have been on glucocorticoids or have already lost BMD, either oral pamidronate or alendronate in oral/intravenous routes can be considered based on the availability.
Misra [67]	Anorexia nervosa[a]	2	Children and adults	55	Bisphosphonates may be considered in adults with osteoporosis, particularly when there is a history of fractures, but should be used cautiously in women of child bearing age.

[a]Review evaluated also other treatments for osteoporosis other than bisphosphonates administration.
[b]4 studies evaluated bisphosphonates administration in a total of 99 patients; the study of Iwasaki *et al.* [68] enrolled 30 patients but did not specify how many received bisphosphonates.

until the achievement of final height [22▪▪,69,70]. If risk factors resolve (mainly in children with secondary osteoporosis) and the patient is clinically stable for at least 6–12 months, discontinuation of bisphosphonates may be considered during growth [22▪▪]. To avoid unnecessary overtreatment, Trejo and Rauch recently proposed to administer intravenous zoledronate in children with osteogenesis imperfecta at a dose that depends on lumbar spine areal BMD *Z*-score results [31▪].

CONCLUSION

Primary and secondary forms of pediatric osteoporosis represent an emerging condition with significant impact on the quality of life. Thus, pediatric osteoporosis should be adequately suspected and properly diagnosed in subjects at risk for fractures. At present, the administration of bisphosphonates represents the main medical treatment of pediatric osteoporosis, but many questions remain unanswered, e.g. optimal route of administration, duration of therapy, long-term safety, possible transplacental passage in women of child-bearing age, efficacy in reducing incident fracture rate. Particularly, accurate selection of children with true indications for bisphosphonates treatment is essential to avoid unnecessary treatment.

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There are no conflicts of interest.

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- ■ of special interest
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Revisiting the role of steroids and aspirin in the management of acute Kawasaki disease

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Purpose of review
Kawasaki disease is an acute multisystem childhood vasculitis with a predilection for the coronary arteries. The role of corticosteroids and acetyl salicylic acid (ASA) in the treatment of acute Kawasaki disease are matters of ongoing debate and changing attitudes from one extreme to the other. Recent work has provided new evidence to guide our thinking about these two therapeutic agents, which will be the focus of this review.

Recent findings
Corticosteroids are effective and well tolerated in Kawasaki disease, both as initial adjunctive treatment in those at high-risk for poor outcome, and as rescue therapy after failed intravenous immunoglobulin (IVIG). Higher doses of ASA (> 30 mg/kg/day) in the acute phase of Kawasaki disease, have no clear benefit over antiplatelet doses in improving coronary outcome.

Summary
Corticosteroids should be used in patients at high-risk for poor coronary outcome, and in patients who fail IVIG. The absence of widely applicable and validated risk-scoring systems in Kawasaki disease outside of Japan remains a limiting factor to identify high-risk children. Current evidence does not demonstrate any advantage of high-dose over low-dose ASA in the acute phase of Kawasaki disease, in preventing coronary artery aneurysms.

Keywords
aspirin, corticosteroids, Kawasaki disease

INTRODUCTION

Kawasaki disease is the leading cause of childhood acquired heart disease in the developed world [1]. Clinically, Kawasaki disease is characterized by prolonged fever for 5 or more days associated with signs of widespread systemic inflammation. The etiopathogenesis of Kawasaki disease reflects a dysregulated immune response to an environmental trigger in a genetically susceptible host. The American Heart Association (AHA) statement on diagnosis and management of Kawasaki disease [2▪], and the Japanese Society of Pediatric Cardiology and Cardiac Surgery (JSPCCS) guidelines [3], provide recommendations to assist physicians caring for patients with Kawasaki disease. The efficacy of intravenous immunoglobulin (IVIG) as a single 2 g/kg infusion to prevent coronary artery abnormality (CAA) during the acute phase of illness is well established and currently standard of care on both sides of the Pacific. The role of corticosteroids and ASA in the acute management of Kawasaki disease remain controversial. The paper will review recent evidence on this topic.

CORTICOSTEROIDS IN KAWASAKI DISEASE

Rationale for use

Corticosteroids are the mainstay of therapy in medium vessel vasculitides with well known and undisputed anti-inflammatory properties.

Case series and retrospective studies

The majority of early reports of corticosteroids in Kawasaki disease are from small case series,

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KEY POINTS

- Corticosteroids are beneficial in reducing inflammation as part of early intensification therapy and rescue treatment after failed IVIG.
- A dose of 2 mg/kg or equivalent of prednisone over an intermediate period of time (>3 days and tapered over 2 weeks) is efficacious and without risk of serious adverse events.
- In the absence of evidence to support the effectiveness of higher doses to prevent adverse coronary outcome, low-dose ASA (3–5 mg/kg) may be used throughout the disease course.
- Predictive scoring algorithms to accurately identify high-risk children from all ethnicities is a priority on our way to improving management and outcome in Kawasaki disease.

nonrandomized studies, and retrospective studies. The first published study on corticosteroids in Kawasaki disease was a small non-randomized study from Japan in 1979 by Kato *et al.* [4]. Five different treatment protocols were assessed for their impact on incidence of coronary artery aneurysms (CAA), three of which included prednisolone (2–3 mg/kg/d) either alone or in combination with warfarin or ASA. The alarmingly higher frequency of CAA (64.7%) in the steroid only group led to a strong hesitation to conduct further studies with corticosteroids. Interestingly, the group that received a combination of prednisolone with ASA, did not report any CAAs, a distinctly different outcome despite the use of prednisolone. In a study of 60 patients, Kijima *et al.* [5] showed that three consecutive daily pulse doses of intravenous methylprednisolone (IVMP) 30 mg/kg/day resulted in improvement in CAA compared to those without steroids. In 1993, Sundel *et al.* [6] reported a small case series of 13 patients with failed IVIG, who were retreated with additional IVIG. Two of these patients remained febrile and benefitted from IVMP (30 mg/kg/day). In 1999, Shinohara *et al.* [7] conducted a retrospective single-center chart review of 299 patients with Kawasaki disease using four regimens including combinations of ASA, IVIG, prednisolone, dipyridamole, and propranolol. They showed significant reduction of fever and CAA incidence in all regimens that included prednisolone. All these early non-randomized studies supported a beneficial role of corticosteroids in Kawasaki disease either as initial therapy or rescue treatment in IVIG failures. A retrospective single-center study by McCrindle *et al.* [8] examined 80 patients with Kawasaki disease with CAA and compared the subgroup that received corticosteroid for duration of fever and evolution of CAAs. Results indicated that the corticosteroid treated group had longer duration of fever, and progressive increase in coronary artery *Z*-scores. Confounding by indication, with more severely affected patients receiving steroids is an important consideration in this retrospective study.

Randomized controlled trials

The first prospective randomized controlled trial (RCT) on corticosteroids as primary therapy in Kawasaki disease came from Boston in 2003 [9], in which patients with Kawasaki disease were randomly allocated to receive IVIG 2 g/kg with ASA 80–100 mg/kg/day or together with the addition of IVMP (30 mg/kg). Patients in the corticosteroid group had no serious adverse events and experienced shorter duration of fever, shorter hospital stay, lower levels of C-reactive protein (CRP); however, no difference in coronary dimensions at week 6. The statistical power of this study was limited with the total number of subjects in each group being less than 25, and the number of patients with coronary aneurysms at week 6 being only 1 in each group.

A larger prospective randomized controlled trial by Inoue *et al.* [10] in 2006, compared CAAs in patients receiving IVIG (1 g/kg/day × 2) with IVIG plus intravenous (IV) prednisolone 6 mg/kg/day, which was switched to oral dosing after fever resolution, and tapered over 15 days after normalization of the CRP. The incidence of aneurysms at 1 month was 3 of 88 in the IVIG alone group and 0 of 90 in the prednisolone plus IVIG group. Secondary outcomes of fever resolution and time to normalization of CRP were also significantly better in the corticosteroid group. Despite limitations (non-blinded echocardiogram assessments and different IVIG infusion regimens), this study suggested that corticosteroids were effective in reducing the incidence of coronary aneurysms, as well as improving clinical and biochemical measures of inflammation. The definite absence of serious adverse effects in the corticosteroid group paved the way for conducting subsequent larger trials.

A multicenter randomized trial by Pediatric Heart Network investigators in 2007 [11] compared the efficacy of a single dose of IVMP with placebo in patients who received IVIG 2 g/kg and ASA at 80–100 mg/kg. They found no difference in coronary artery dimensions at weeks 1 and 5. Secondary outcomes including rate of retreatment with IVIG, number of days of hospitalization, and fever were also comparable, suggesting no additional benefit with corticosteroids. Interestingly, a post-hoc subgroup analyses of children with persistent fever

requiring retreatment with IVIG showed that coronary outcomes were better in the initial corticosteroid treated group, suggesting that corticosteroids may be beneficial in this high-risk group.

Early intensification of treatment

Recent evidence for the efficacy of corticosteroids in improving coronary outcome in Kawasaki disease comes from the RAISE trial [12▪▪] which included 74 Japanese centers. A computer-generated randomization sequence divided the cohort into two equal groups of which 125 patients received prednisolone in addition to standard of care (IVIG and ASA), and 123 patients received only standard of care. Prednisolone was initiated at 2 mg/kg intravenously for 5 days or until defervescence, then switched to the oral route and tapered over 15 days after CRP normalization. Interim analysis demonstrated a statistically significant difference in the incidence of coronary aneurysms between the two groups, leading to premature termination of the study. The primary outcome of incidence of CAAs was significantly lower in the corticosteroid group, without any difference in the incidence of serious adverse events. Of the secondary outcomes, the need for additional rescue treatment was higher in the group without corticosteroids. Interestingly, the cumulative dose of prednisolone in this trial was higher compared to previous studies that used a single dose of pulse methylprednisolone and failed to demonstrate differences in coronary artery outcomes. Notably, the RAISE study focused on children at high-risk for poor coronary outcome and included only patients with a Kobayashi score of 5 points or higher and a much longer total duration of corticosteroids. Risk stratification is used routinely in Japan, where risk-scores are effective and validated. Unfortunately, the Kobayashi score (and other scoring algorithms) are not sensitive outside of Japan. This study provides the best evidence for a beneficial role of corticosteroids on coronay outcome in Kawasaki disease, with a focus on those at high-risk for poor coronary outcome.

Chen *et al.* [13] conducted a meta-analysis of nine clinical studies from Japan and North America, with 1011 patients in total that compared the efficacy of IVIG plus corticosteroids with IVIG alone. All studies used corticosteroids as early initial treatment, albeit in different doses and regimens. The combined results showed that adding corticosteroids significantly reduced the risk of CAA [odds ratio (OR) = 0.3; 95% confidence interval (CI), 0.20–0.46]. A subgroup analyses of the RCTs (OR = 0.3; 95% CI, 0.18–0.5), focused on high-risk patients (OR = 0.2; 95% CI, 0.1–0.36) and studies with blinded-endpoints (OR = 0.32; 95% CI, 0.19–0.55) yielded similar results. There was no significant difference in the incidence of severe adverse events between the groups with and without steroids. This meta-analysis suggested a substantial benefit of early corticosteroid use, although the large variability in the regimens used in individual studies precluded a conclusion about the ideal corticosteroid dosing schedule.

The potential for targeted use of corticosteroid for refractory Kawasaki disease was further explored by Kimura *et al.* [14]. A multicentre, prospective nonrandomized, nonblinded study with 1087 patients, which divided them into high and low CRP groups after IVIG failure. The high CRP group received an intensified regimen with the addition of prednisolone to the second IVIG dose. The combination of IVIG and corticosteroids was more effective in abating fever in this group, as compared to IVIG alone (81.3 vs 67.3%). The results of this study re-emphasized that the incidence of CAA is high (18.8%) in IVIG failures and early intensification or retreatment for this group including the use of corticosteroids is prudent.

A recent Cochrane review [15▪] concluded that the use of corticosteroids in the acute phase of Kawasaki disease reduces the incidence of CAA, duration of fever, time for normalization of CRP, and length of hospitalization. The greatest benefit of corticosteroids was in Japanese children, but the use of different treatment regimens may have led to the different outcomes in Japanese and American studies. This paper reiterated that corticosteroids are most beneficial in children with high-risk scores and the benefit increases with a prolonged course, versus a single dose. The timing of corticosteroid administration was further explored by Chen *et al.* [16▪▪] in another meta-analysis of 16 studies, including the nine from the previous meta-analysis [13], with 2746 patients in total, in whom corticosteroids were either administered at the onset, or as rescue therapy after IVIG failure. The incidence of CAA was lower in those who received corticosteroid versus the IVIG only group (OR = 0.424; 95% CI, 0.270–0.665). Greater benefit was seen in the subgroup that received early intensification with corticosteroids compared to the IVIG only group, but no difference was observed in the corticosteroid rescue therapy group compared to IVIG. Suggesting that favorable effects of corticosteroid were higher when given early, without an increased risk of adverse events. This led the authors to conclude that 'corticosteroids most likely exert a beneficial effect when initiated at the diagnosis of Kawasaki disease rather than after the failure of initial IVIG therapy'. However, the meta-analysis only included 6 re-treatment

studies with 383 patients, of which 167 were treated with steroids in varying doses and regimens. As is the case with most meta-analyses, the heterogeneity of treatment protocols and study designs imposes limitations on interpretation. In the absence of well designed randomized controlled trials, the data point to corticosteroids being beneficial in Kawasaki disease, more so if given early than late in the disease course, and most certainly do no harm [17▪]. Steroids effectively target inflammation and increasingly appear to improve coronary outcome in Kawasaki disease. Recent data support the approach of early intensification of therapy with addition of corticosteroids to current standard of care in high-risk children in addition to inclusion as part of the re-treatment protocol in those with IVIG failure. The challenge is accurate risk stratification, with identification of children at high-risk for poor coronary outcome in all ethnicities a priority. Even though the ideal dose and schedule remains to be established, it is clear from the data that intravenous or oral dose of corticosteroids equivalent to prednisone at 2 mg/kg in the acute phase in short to medium-term regimens (more than 3 days) followed by a tapering schedule dictated by clinical and laboratory signs of inflammation over 2 weeks is effective and does not pose additional safety concerns.

ASPIRIN AND Kawasaki disease

Rationale for use

Acetylsalicylic acid (ASA/aspirin) is a non-steroidal anti-inflammatory drug that has been used for several decades in Kawasaki disease. In high doses, it exerts an anti-inflammatory effect, and in lower doses, it has antithrombotic effects [18]. According to recent AHA guidelines, moderate (30–50 mg/kg/day) to high-dose (80–100 mg/kg/day) ASA is recommended in the acute phase of Kawasaki disease. The JSPCCS guidelines recommend 30–50 mg/kg/day of ASA in the febrile phase. ASA was the most widely used anti-inflammatory therapy for Kawasaki disease before IVIG was established as standard treatment. In the post-IVIG era however, there has been considerable debate about the optimal dose of ASA in the acute phase of Kawasaki disease and whether there is a role in preventing CAA. Anti-inflammatory steady-state levels are often difficult to achieve in patients with Kawasaki disease, owing to decreased gastrointestinal absorption and increased renal clearance of ASA during the acute phase of disease. Hypoalbuminemia in the acute phase decreases protein binding of the active form of the drug, and may lead to therapeutic effects or even toxic effects at lower total ASA levels [19].

Early literature

A potential limitation in interpretation of early literature is the use of variable regimens and doses of IVIG. An early meta-analysis of 28 non-randomized studies on the efficacy of different doses of ASA and IVIG, by Durongpisitkul *et al.* [20] showed that there was no additional benefit of high-dose ASA to IVIG in preventing CAA. Furusho *et al.* in 1991 [21], randomized 102 children to receive IVIG alone (200 mg/kg/day for 5 days) or IVIG with ASA (30–50 mg/kg/day until defervescence), with no clear benefit of adding ASA to IVIG on the rate of CAAs. Shulman *et al.* [22] reviewed six U.S. and Japanese multicenter RCTs comparing the effect of various doses of IVIG with ASA on the rate of CAA development in Kawasaki disease. A total of 868 Japanese patients were treated with moderate dose (30–50 mg/kg per day), and 761 U.S. patients treated with high-dose ASA (80–120 mg/kg/day), with total IVIG dose ranging from 1 to 2 g/kg. The results indicated that the incidence of CAAs was inversely related to the total dose of IVIG but independent of ASA dose. These studies point to a limited role of ASA in reducing CAA in the post-IVIG era.

Recent literature

Several nonrandomized retrospective studies comparing ASA dosing brought to light the limited usefulness of higher doses of ASA in prevention of CAA. In 2004, a group of Taiwanese researchers [23] retrospectively studied 162 patients with Kawasaki disease, all of whom received IVIG 2 g/kg as a single infusion without concomitant ASA treatment. The IVIG resistance rate of 5.56% was similar to that reported in studies with combined IVIG and ASA, suggesting that ASA in the acute phase of Kawasaki disease had no effect on preventing IVIG failure. A Cochrane review on salicylate use in Kawasaki disease [24] identified a single RCT on ASA dosing. The authors were unable to recommend the optimal dose of ASA, because of scarcity of data from good quality RCTs directly comparing high-dose (80–100 mg/kg/day) with low-dose (3–5 mg/kg/day) ASA. In a recent review from the United Kingdom [25] an ASA dose of 30–50 mg/kg/day was recommended based on interpretation of results from Shulman's meta-analysis.

More recently, a small retrospective study from Israel [26], comparing high dose (80–100 mg/kg/day) with low dose ASA (3–5 mg/kg/day) and IVIG (2 g/kg), found no significant difference between the two groups in the rate of occurrence of CAAs. A much larger study using data from the eighth nationwide survey on Kawasaki disease from Korea [27▪▪] had 8546 patients who were divided into two

groups according to the dose of ASA (>30 or 3–5 mg/kg/day). The prevalence of CAA based on *Z*-score (24.8 vs. 18.3%; $P = 0.001$) and on the Japanese Ministry of Health criteria (19.0 vs. 10.4%; $P < 0.001$) was significantly higher in the 7947 patients who received medium-dose or high-dose ASA compared with the 509 patients who received low-dose ASA. In addition, the use of high-dose of ASA was a significant predictor of CAA by logistic regression analysis, thus suggesting that intermediate and high-dose ASA is not protective against CAA, and perhaps even a risk factor for development of CAAs. This is in accord with laboratory data showing the paradoxical effect of intermediate-concentrations ASA on enhancing pro-inflammatory cytokine production [28]. Despite being a retrospective cross-sectional survey, this is by far the largest cohort of patients with Kawasaki disease that addresses a very relevant clinical question regarding optimal ASA dose, and the results indicate limited usefulness and potential harm from higher doses of ASA.

In the absence of controlled data, the optimal dose of ASA in the acute phase of Kawasaki disease remains debated. However, there is sufficient evidence to suggest that higher doses (>30 mg/kg/day) have no clear advantage in suppressing inflammation, and may increase the risk of not only adverse effects but also poor coronary outcome, suggesting that antiplatelet doses of ASA (3–5 mg/kg/day) may be the most rational, 'do no harm' approach, until better evidence becomes available.

CONCLUSIONS

Corticosteroids given systemically either IV or oral at the equivalent of prednisone 2 mg/kg/day for a short to intermediate course (> 3 days) followed by a tapering schedule over 2 weeks as dictated by clinical and laboratory measures of inflammation is well tolerated and effective in controlling clinical signs of inflammation. Early addition of steroids with intensification of treatment is most effective, especially in those at high-risk for poor outcome, but is predicated on the need for accurate predictive scoring systems to identify high-risk children. This re-emphasizes the need to develop more widely validated risk-stratification algorithms.

The optimal dose of ASA in the acute phase of Kawasaki disease is less clear; however, recent data does not support any advantage of medium or high-dose ASA in lowering the risk for development of CAA or reducing clinical features of inflammation. In fact, the opposite may be true with higher risk of poor coronary outcome in those on medium or high-dose ASA. Thus, using low-dose ASA (3–5 mg/kg) throughout the course of Kawasaki disease may be the safest option until appropriately designed and sufficiently powered studies can answer this question.

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Conflicts of interest

There are no conflicts of interest.

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