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A Narrative Review on Integrative Bioinformatics Approaches for microRNA Research in Familial Mediterranean Fever: Current Insights and Future Directions

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ABSTRACT

Background and Aims: Familial Mediterranean Fever (FMF) is a monogenic autoinflammatory disease caused by mutations in the *MEFV* gene, resulting in recurrent inflammatory episodes and a risk of developing amyloidosis. Although its pathophysiology is well described, FMF still lacks specific biomarkers and personalized treatment strategies. MicroRNAs (miRNAs), which regulate gene expression posttranscriptionally, have emerged as promising diagnostic, prognostic, and therapeutic biomarkers. Given their complex expression patterns, bioinformatics approaches are essential for their identification and interpretation. Hence, this review aims to summarize current bioinformatics tools used in FMF-related miRNA research and highlight additional platforms employed in other inflammatory diseases that may advance FMF research.

Methods: A narrative review was conducted by examining published FMF studies that applied miRNA-focused bioinformatics analyses, including miRWalk, TargetScan, and machine learning pipelines. To identify tools with potential relevance to FMF, platforms widely used in rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, and psoriasis, such as miRDeep2, miRTarBase, DIANA-miRPath, miEAA, and MAGPIE, were evaluated for their analytical strengths and applicability to autoinflammatory pathways.

Results: Most FMF studies rely on a narrow set of tools, primarily miRWalk or TargetScan for target prediction. Emerging machine learning approaches have also been utilized to classify patients and explore candidate biomarkers. Other inflammatory diseases use more advanced platforms enabling miRNA discovery, validated interaction mapping, pathway enrichment, and multi-omics integration. Tools such as miRDeep2, miRTarBase, DIANA-miRPath, miEAA, and MAGPIE remain underutilized in FMF. Key limitations include small cohorts, patient heterogeneity, and limited experimental validation.

Conclusion: Broadening the bioinformatics toolkit for FMF miRNA research could significantly enhance biomarker identification and mechanistic insight. Larger datasets, integrated analysis pipelines, and cross-disciplinary collaboration are essential to advancing precision diagnostics and targeted therapies for FMF.

Abbreviations: AI, artificial intelligence; CAPS, cryopyrin-associated periodic syndrome; CDS, coding sequence; ceRNA, competing endogenous RNA; circRNA, circular RNA; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; DIRA, deficiency of the interleukin-1 receptor antagonist; ESR, erythrocyte sedimentation rate; FMF, familial Mediterranean fever; GO, gene ontology; GSEA, gene set enrichment analysis; IBD, inflammatory bowel disease; IL, interleukin; lncRNA, long noncoding RNA; IPA, ingenuity pathway analysis; KEGG, Kyoto encyclopedia of genes and genomes; MAGPIE, meta-analysis gene-set enrichment and pathway integration environment; MCODE, molecular complex detection; MEFV, Mediterranean fever gene; miEAA, microRNA enrichment analysis and annotation; miRNA, microRNA; ML, machine learning; mRNA, messenger RNA; MTIs, microRNA–target interactions; OA, osteoarthritis; ORA, over-representation analysis; qPCR, quantitative real-time polymerase chain reaction; RA, rheumatoid arthritis; SAA, serum amyloid A; SLE, systemic lupus erythematosus; TNF, tumor necrosis factor; TRAPS, tumor necrosis factor receptor-associated periodic syndrome; UC, ulcerative colitis; UTR, untranslated region.

1 | Background

Familial Mediterranean Fever (FMF) is a monogenic autoinflammatory disease most common in populations around the Mediterranean Sea. The disease usually begins in childhood or adolescence and is marked by recurrent episodes of fever, serositis, arthritis, and systemic inflammation [1, 2]. Daily colchicine remains the primary therapy for FMF, effectively reducing the frequency and severity of attacks as well as the risk of amyloidosis, the most severe complication of the disease [3, 4]. For patients who are intolerant or resistant to colchicine, interleukin-1 (IL-1) inhibitors offer an effective alternative [5].

FMF is caused by mutations in the *MEFV* (MEditerranean FeVer) gene, which is located on chromosome 16p13.3 [6]. This gene comprises 10 exons and encodes pyrin, a cytosolic protein composed of 781 amino acids. Pyrin acts as a key regulator of the inflammasome, a multiprotein complex involved in the activation of caspase-1 and the production of pro-inflammatory cytokines, mainly IL-1β, and IL-18 [7, 8]. FMF mutations impair the phosphorylation-dependent regulation of pyrin, resulting in inappropriate inflammasome assembly, caspase-1 activation, and excessive secretion of IL-1β and IL-18. This leads to ongoing subclinical inflammation, which drives the recurrent inflammatory episodes characteristic of FMF [1, 3]. More than 400 *MEFV* variants have been identified to date, with M694V, M694I, M680I, and V726A among the most pathogenic and prevalent alleles [9, 10].

The diagnosis of FMF is primarily clinical, based on characteristic features of the disease. It is often supported by a relevant ethnic background, a positive family history, and a favorable response to colchicine therapy [11]. Since the identification of the *MEFV* gene in the late 1990s, molecular testing has become central to FMF diagnosis and has helped reduce delays in treatment [12, 13]. Additionally, laboratory markers of systemic inflammation, such as leukocyte count, erythrocyte sedimentation rate, C-reactive protein, serum amyloid A, and fibrinogen levels, are often assessed, particularly during acute attacks, to support the clinical diagnosis [14]. However, some patients who fulfill clinical criteria, despite having only one detectable *MEFV* mutation, may still develop FMF symptoms, underscoring the complexity of genotype–phenotype correlations and the limitations of genetic testing alone [15]. Moreover, the wide variability in clinical presentation, attributed to *MEFV* allelic heterogeneity, modifier genes, and epigenetic modifications, challenges the development of standardized diagnostic approaches for FMF and may impact disease severity and treatment response [16–18].

Considering these limitations, there is growing interest in identifying novel molecular markers that can improve FMF diagnosis. One such promising class of biomarkers is microRNAs (miRNAs). miRNAs are small, noncoding RNA molecules, typically 16–24 nucleotides in length [19]. Through sequence-specific interactions with messenger RNAs (mRNAs), miRNAs regulate gene expression posttranscriptionally, thereby modulating key inflammatory and immune pathways implicated in FMF [20]. Recent studies have demonstrated that miRNA expression profiles are altered in FMF patients during both attacks and remission. For instance, miR-146a, miR-155, and miR-4520a have been reported to modulate inflammasome and innate immune pathways, and to directly interact with *MEFV* transcripts [21, 22]. Their dysregulation implies that specific miRNAs could serve as noninvasive biomarkers for diagnosis, disease monitoring, or treatment response [19, 23].

miRNA data analysis presents notable challenges. The expression profiles are complex, highly context-dependent, and often involve large-scale datasets generated by high-throughput platforms [24, 25]. To accurately interpret this information, the application of bioinformatics tools is crucial. These tools enable the detection and profiling of miRNA in biological samples, prediction of miRNA–mRNA interactions, and their mapping to relevant biological pathways and functions [26]. Originally developed for oncology and systemic immune diseases, some of these platforms are now being adapted for FMF research, enhancing understanding of its molecular mechanisms and aiding in identifying novel diagnostic markers and therapeutic targets [27]. Nevertheless, their application to FMF-miRNA datasets remains limited, constraining FMF-specific conclusions.

This review highlights an important gap in FMF research: the limited use of bioinformatics tools for miRNA analysis. To address this, we examined tools currently employed in FMF-miRNA studies, such as miRbase, TargetScan, miRWalk, and custom machine learning (ML) pipelines, and identified additional computational resources successfully used in other diseases but underutilized in FMF. Broader adoption of these tools could advance miRNA-based analyses and facilitate the discovery of FMF-specific biomarkers.

2 | Methodology

A comprehensive literature search strategy was employed across various databases, such as PubMed and Google Scholar, using combinations of the following keywords: “Familial Mediterranean Fever” or “FMF” and “microRNA” or “miRNA” and “bioinformatics” or “bioinformatics tools” or “miRNA profiling” or “target prediction” or the names of specific tools. Only peer-reviewed studies published in English were included. Priority was given to research focusing on FMF-specific miRNA profiles, studies utilizing tools directly applied to FMF datasets, and analyses of *MEFV* gene regulation mediated by miRNAs. Given the limited number of FMF-specific miRNA studies, additional literature from related autoinflammatory or autoimmune conditions was incorporated, along with general miRNA bioinformatics research, when relevant methodologies could be adapted to FMF. The included publications comprised original research articles, methodological tool papers, and official software documentation, providing a comprehensive overview of applicable approaches and resources. The screening process was carried out in two stages: initially, by reviewing titles and abstracts to assess topic relevance, and subsequently through full-text evaluation to confirm eligibility for inclusion. The selected studies were then assessed based on the bioinformatics methods used, their functional scope, and their relevance and applicability to FMF research.

3 | Bioinformatics Tools Employed in FMF Research

This section reviews key bioinformatics platforms that have contributed to FMF-miRNA research, with emphasis on

database and annotation, miRNA profiling, target prediction, and ML (Table 1).

3.1 | Database and Annotation Tools

miRBase has served as the main registry and database for miRNAs since 2002. It assigns official names to newly reported miRNAs and archives their sequences, hairpin precursors, and genomic positions while linking each record to relevant studies and other resources [89]. By combining standardized nomenclature with comprehensive sequence data and cross-references to predicted targets, miRBase remains the leading online resource for miRNA research [28].

In FMF studies, miRBase is mainly used to obtain validated sequences and standardized gene names for experimental design rather than as an analytical platform [28]. The latest release catalogs approximately 2600–2656 mature human miRNAs, which may regulate up to 60% of protein-coding genes [29]. Beyond FMF, miRBase is a foundational resource in studies of rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and inflammatory bowel disease (IBD), where it helps compile lists of known miRNAs, circular RNAs (circRNAs), and long noncoding RNAs (lncRNAs) for constructing interaction networks and assessing expression changes. For instance, investigators studying RA assembled these databases to build a co-expression network comprising 228 circRNA–miRNA links [31], while workflows in SLE and IBD rely on miRBase for consistent annotation of miRNA sequences, enabling network visualization and functional enrichment analyses [31].

miRBase offers many advantages, including the broad coverage of hairpin and mature miRNA sequences across many organisms, with each entry accompanied by its genomic location and links to relevant literature [28]. As the official naming authority for newly identified miRNAs, it ensures consistent nomenclature among researchers. The database also integrates deep sequencing data and provides high-confidence annotations supported by billions of reads, while offering links to external target prediction resources [28]. Despite these strengths, the quality of entries can vary, as most submissions are author-provided and undergo minimal curation [28]. Moreover, frequent updates may lead to changes in definitions and annotations, which can create inconsistencies across databases and publications, necessitating the use of conversion tools to translate names and accession numbers between releases [32].

3.2 | Target Prediction Tools

3.2.1 | *miRWalk*

miRWalk is an open-access, integrative bioinformatics tool that predicts miRNAs' binding sites across entire gene sequences, including the coding sequence (CDS) and 3′ and 5′ untranslated regions (UTRs). Unlike other tools that focus only on the conserved 3′ UTR seed region, miRWalk covers regions often overlooked, enabling more comprehensive detection of miRNA–gene interactions relevant to complex diseases [33, 34].

No study has yet employed miRWalk exclusively for FMF. However, FMF was included in a broader project established by Akbaba et al., involving multiple autoinflammatory diseases. In this study, miRWalk, along with TargetScan and miRBD, was used to predict the targets of miR-30e-3p in patients with FMF, cryopyrin-associated periodic syndrome (CAPS), and tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS), highlighting the potential of this tool for FMF target prediction [35]. Beyond FMF, miRWalk has been applied to predict miRNAs-mRNAs regulatory links in SLE and endometriosis, leading to the identification of common diagnostic markers and pathways [36]. It has also been used to identify target genes of miR-16-5p, miR-21-5p, and miR-155-5p in circulating vesicles as potential biomarkers for psoriasis [37], and to determine the regulatory target of miR-34a-3p in RA, which was subsequently validated via a luciferase reporter assay [38]. These examples demonstrate that miRWalk is not limited to predictions alone but can also facilitate biological discoveries in inflammatory conditions when combined with experimental validation.

miRWalk is a powerful tool because it integrates both predicted and experimental data from different databases such as miRanda, TargetScan, and RNA22, and includes information from over 12 species [34]. This versatility makes miRWalk valuable for generating research hypotheses. Despite its benefits, miRWalk has some downsides. Its predictions are primarily based on sequence complementarity, which may not always reflect *in vivo* conditions. Additionally, it does not account for temporal and spatial factors, such as tissue-specific expression or disease context, potentially leading to biologically irrelevant predictions. These limitations underscore the importance of experimental validation to confirm the functional relevance of the predicted interactions [34]. In conclusion, although miRWalk is not commonly used in FMF, it holds significant potential to identify candidate miRNA targets, particularly when combined with complementary experimental approaches.

3.2.2 | *TargetScan*

TargetScan is a widely used miRNA target prediction tool that identifies possible matches between the miRNA seed region and conserved sequences within the 3′ UTR of target genes [39]. Unlike broader tools like miRWalk, TargetScan specifically emphasizes evolutionarily conserved 3′ UTR binding sites. Its scoring system incorporates various factors, including binding site accessibility, evolutionary conservation, and transcript abundance, allowing researchers to prioritize interactions based on likely biological significance [39].

In FMF research, TargetScan was used indirectly by Akbaba et al., who investigated miR-30e-3p in patients with different systemic autoinflammatory diseases. Using TargetScanHuman, they identified various inflammatory genes potentially regulated by miR-30e-3p [35]. This approach has also gained popularity in studying other inflammatory conditions, such as RA, where predicted targets of miR-34a-3p were confirmed with luciferase assays [38]. It was also employed in SLE to analyze cooperative roles of miRNAs with similar seed regions [40], and in ulcerative colitis (UC) to identify NF-κB pathway targets of miR-125b and miR-223 [41].

A major advantage of TargetScan is its focus on conserved targets, enhancing prediction reliability and functional relevance in vivo [39]. Additionally, TargetScan utilizes 3P-seq data to pinpoint exact miRNA binding sites within cells, combining evolutionary and experimental evidence for trustworthy

TABLE 1 | Overview of bioinformatics tools employed in FMF and other inflammatory diseases: applications, advantages, and limitations.

Tool	Type of tool	Purpose/use	Application in FMF	Applicability in other inflammatory diseases	Advantages	Limitations
miRBase	Database and annotation tool	A comprehensive miRNA registry and database used for storing sequences, hairpin precursors, and genomic locations of miRNAs [28]	Applied in FMF as a reference for known miRNAs, serving as a source for standardized gene names and sequences, but not for analysis [29, 30]	Key for miRNA research in RA, SLE, and IBD, used to compile miRNA and RNA lists for network construction [31]	• Extensive miRNA sequence coverage • Includes genomic locations and literature links • High-confidence annotations with deep-sequencing data • Consistent miRNA naming [28]	• Varies in quality due to minimal curation [28] • Frequent updates cause version inconsistencies [32] • Requires conversion tools between releases [32]
miRWalk	Target prediction	Predicts miRNA binding across coding regions and UTRs for broad miRNA-gene interaction coverage [33, 34]	Used in a multi-autoinflammatory disease study, including FMF to predict miR-30e-3p targets [35]	Used in RA, SLE, psoriasis, and endometriosis to predict miRNA–mRNA interactions, identify diagnostic markers, and explore inflammation-related regulatory pathways [36–38]	• Scans full gene sequence • Merges multi-database data • Spans 12+ species • Aids in hypothesis generation [34]	• It may not reflect in vivo conditions and overlooks gene expression context • Requires experimental validation [34]
TargetScan	Target prediction	Predicts conserved miRNA binding sites, primarily in 3′ UTRs, based on evolutionary conservation [39]	Applied indirectly in an autoinflammatory disease research, including FMF to identify potential inflammatory genes regulated by miR-30e-3p [35]	Used in RA, SLE, UC, and other inflammatory diseases to predict conserved miRNA targets within inflammatory pathways and validate key miRNA–mRNA pairs via luciferase assays [38, 40, 41]	• Reliable due to evolutionary conservation • Highly accurate for conserved targets • Uses 3P-seq data to improve site accuracy [35, 39]	• Limited to 3′UTR • Misses noncanonical targets • Needs more data to further support predictions [39]
MiRDeep2	Detection and profiling tools	Identifies known and novel miRNAs from deep sequencing data, including noncanonical miRNAs [42]	No	Used in SLE, psoriasis, COVID-19, and other autoimmune diseases to detect novel miRNAs from small RNA-seq data and identify disease-specific signatures linked	Identifies known and novel miRNAs [46]	• Overcounts reads • Cannot detect indels [46]

(Continues)

TABLE 1 | (Continued)

Tool	Type of tool	Purpose/use	Application in FMF	Applicability in other inflammatory diseases	Advantages	Limitations
				to immune dysregulation [43–45]		
miRDB	Target prediction	Uses ML to predict miRNA targets based on experimental data [47]	No	Applied in RA, SLE, and other diseases to predict inflammation-related gene targets and miRNAs acting as potential therapeutic biomarkers [48, 49]	• ML approach based on experimental data • Provides functional weighing for targets [50]	• High number of false positives • Requires data filtering and validation [51]
miRTarBase	Target prediction/ validation database	Provides experimentally validated miRNA-target interactions through qPCR and reporter assays [52]	No	Used in RA, SLE, and other diseases to validate experimentally confirmed miRNA–mRNA interactions [53, 54]	• Experimentally validated interactions • Useful for confirming predictions [55]	Missing many known miRNA targets [56]
DIANA-miRPath	Pathway analysis	Analyzes miRNA–pathway interactions using KEGG and GO data to identify affected biological processes [57]	No	Used in RA, SLE, psoriasis, and COVID-19 for pathway enrichment, linking dysregulated miRNAs to cytokine and immune signaling cascades [57–60]	Real-time pathway enrichment [61]	Biased results with random miRNA sets [62]
IPA	Pathway and functional analysis	Identifies key regulators and pathways from gene expression data, linking them to disease mechanisms [63]	No	Applied in RA, lupus, psoriasis, and UC to identify upstream regulators and canonical pathways involving cytokines [64–66]	Curated pathway and network analysis [67]	• Limited species involved and missing novel interactions • Limited access and transparency [68]
miEAA/ GSEA	Pathway and functional analysis	Performs enrichment analysis of miRNA sets, linking them to biological processes like immune activation or inflammation [69]	No	Used in OA and cardiovascular inflammation to enrich miRNA sets linked to apoptosis, cytokines, and immune activation pathways [70, 71]	Broad functional annotation for miRNA [62]	Limited rare species coverage [69]

(Continues)

TABLE 1 | (Continued)

Tool	Type of tool	Purpose/use	Application in FMF	Applicability in other inflammatory diseases	Advantages	Limitations
MAGPIE	ML	Uses ML algorithms to predict the pathogenicity of genetic variants based on multiple annotations [72]	No	Used in genomic studies of Mendelian diseases to predict pathogenic variants [72]. Not yet used in inflammatory diseases	• High predictive accuracy, especially for rare variant prediction • can be applied to many types of genomic variant analyses [72]	• Less effective recognition of complex diseases • Limited clinical validation • Slower runtime [72]
Custom ML pipelines	ML	Integrates multiple data types (genetic, clinical, transcriptomic, etc.) to design tailored analytical models [73–75]	Employed to predict colchicine resistance, assist diagnosis, evaluate expert reliability, and classify variant origins [76–79]	Applied in RA, SLE, COVID-19, and psoriasis for disease classification, flare prediction, and expression pattern using integrated omics data [80, 81].	• Flexible • Scalable • Combines clinical and omics data • Fast in detecting patterns [82]	• Limited explainability • Bias from unequal data distribution classes • Not widely used in clinics [83, 84]
Cytoscape	Network visualization and analysis tool	Visualizes biological networks, overlays gene-expression data, and links to functional annotation databases [85]	No	Widely used in RA for building ceRNA and circRNA–miRNA networks [31, 86]	• Modular design for tailored analysis [87] • Over 250 plug-ins for various tasks [87] • Integrates expression data [85]	• Can overwhelm memory with large networks • Steep learning curve [88]

Abbreviations: ceRNA, competing endogenous RNA; circRNA, circular RNA; COVID-19, coronavirus disease 2019; FMF, familial Mediterranean fever; GO, gene ontology; IPA, ingenuity pathway analysis; KEGG, Kyoto encyclopedia of genes and genomes; lncRNA, long noncoding RNA; miRNA, microRNA; ML, machine learning; OA, osteoarthritis; qPCR, quantitative real-time polymerase chain reaction; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; UTR, untranslated region.

predictions [35]. However, it has limitations: it primarily focuses on the interactions within the 3′ UTR and may overlook potential binding sites in 5′ UTRs or coding regions [39]. Furthermore, it does not account for tissue-specific gene expression or gene activity under different conditions [39]. In summary, TargetScan remains a valuable resource for miRNA target prediction. Its previous use in FMF and other inflammatory cohorts suggests it can continue to support FMF research, particularly when combined with functional assays or complementary tools such as miRWalk or miRTarBase [35, 38].

3.3 | ML and Integrative Tools

3.3.1 | Custom ML Pipelines

Custom ML pipelines are tailored computational workflows designed to address specific challenges in biomedical research [73]. These pipelines integrate several types of data, including genetic, transcriptomic, and clinical data, to facilitate tasks such as disease classification, severity assessment, and biomarker discovery [74]. By combining omics data with clinical insights, ML models support personalized strategies for diagnosis and therapy [75].

In FMF, ML has been used to aid diagnosis, predict treatment responses, and elucidate patterns of disease distribution. For example, ML has been used to classify the origin of *MEFV* gene variants, revealing variant distributions and ethnic associations [76], identify patients resistant to colchicine for tailored treatment [90], and distinguish FMF from other conditions, such as deficiency of the IL-1 receptor antagonist, using generative artificial intelligence (AI) [77]. It was also used in a multi-center study to validate AI-assisted diagnosis in pediatric FMF [78]. Beyond FMF, ML has advanced research in other inflammatory and complex diseases [80]. In SLE, ML integrated gene expression data to predict flares with 83% accuracy rate [80]. Random forest-based ML was also used in SLE to identify disease-specific gene expression patterns [73]. In RA, ML predicted treatment responses and classified patients based on synovial gene expression [91]. More recently, deep-learning pipelines have been developed to analyze skin images in psoriasis patients [92].

Custom ML pipelines offer numerous benefits, including flexibility, efficiency, and scalability. They enable integration of clinical and omics data, faster analysis, and recognition of patterns in large datasets. However, challenges remain regarding data interpretation [83], potential biases stemming from unequal representation of classes, and limited integration into routine clinical practice [84]. In conclusion, custom ML pipelines hold great promise for advancing FMF and other inflammatory conditions research, supporting diagnosis, treatment planning, and disease monitoring, provided their application is ethical, validated, and integrated into healthcare workflows.

4 | Bioinformatics Tools with Potential Applications in FMF

4.1 | Detection and Profiling Tools

miRDeep2 is a computational tool utilized to accurately detect and quantify both known and novel miRNAs from deep sequencing data. It is valuable for documenting changes in miRNA expression across various conditions. In SLE research, miRDeep2 has helped identify miRNAs that are differentially expressed between diseased and healthy tissues [43]. Similarly, using miRDeep2 for small RNA analysis in patients with SLE and psoriasis has revealed novel miRNAs associated with these diseases [44]. This wide adoption allowed miRDeep2 to gain popularity and become widely recognized [46]. Nevertheless, miRDeep2 has technical limitations, such as over-counting sequence reads and difficulty detecting variants with insertions or deletions (indels) [46].

4.2 | Target Prediction Tools

miRDB is an online resource for predicting miRNA targets and elucidating their functions [47]. Its strength lies in the use of MirTarget, an ML algorithm that uses a support vector machine trained on validated sequencing results to predict targets and assign functional relevance [48, 50]. miRDB has been applied in RA and SLE to identify miRNA-regulated genes involved in inflammation, uncovering candidates with potential as therapeutic targets or disease biomarkers [48, 49]. A limitation of miRDB is the large number of predicted targets, leading to false positives, hence necessitating data filtering and validation [51].

On the other hand, miRTarBase provides experimentally confirmed miRNA–target interactions (MTIs) using methods such as qPCR and reporter assays [52]. With over 3.8 million validated MTIs, it is one of the most comprehensive resources for confirmed miRNA targets [93]. In SLE, miRTarBase was employed to construct miRNA–mRNA networks and integrate experimental validation to ensure reliability [94, 95]. It is also frequently used in RA to validate MTIs involved in cytokine production and immune cell activation [53, 54]. Overall, miRTarBase offers a reliable, experimentally validated database that enhances the credibility of bioinformatics analyses [55]. Its main limitation is incomplete coverage, as many known interactions are omitted [24].

4.3 | Pathway and Functional Analysis Tools

DIANA-miRPath is an online platform that compiles multiple databases and miRNA target prediction algorithms, such as microT-CDS and TarBase/miRTarBase, along with Kyoto encyclopedia of genes and genomes (KEGG) and gene ontology (GO) data, to identify pathways affected by a set of miRNAs [57]. It has been widely used in immune-mediated disease research to interpret the functional impact of miRNA changes. In SLE, DIANA-miRPath revealed that immune and inflammatory pathways in KEGG were enriched by disease-associated miRNAs [57]. In RA, it identified pathways such as cytokine signaling and T cell receptor pathways affected by targets of differentially expressed miRNAs. Similarly, in psoriasis, the tool has been employed to explore miRNA targets and their association with key cellular processes such as adhesion, migration, and proliferation, hinting at their relevance in psoriasis development and progression [58]. DIANA-miRPath performs pathway enrichment analyses for miRNAs across multiple species using integrated data and robust statistical methods [61]. However, enrichment results may be biased when based solely

on predicted targets, as random miRNA sets can yield false-positive associations [62].

Ingenuity pathway analysis (IPA) is a commercial tool that contextualizes gene expression, proteomics, or miRNA data within critical biological networks and pathways [63]. By analyzing gene lists or expression profiles, IPA identifies enriched canonical pathways, network associations between genes, and upstream regulators. This tool is frequently used in autoimmune disease research, particularly transcriptomic and proteomic studies. In RA, IPA revealed pathways linked to inflammation and pain by uncovering networks of genes associated with immune responses and cytokine regulation [64]. In UC and psoriasis, it facilitated the discovery of key pathways involving interferon and TNF, as well as master regulators such as transcription factors and cytokines, linking molecular information to known disease mechanisms [65]. In SLE, IPA clarified gene expression profiles and highlighted key regulatory pathways [66]. IPA's strengths include a curated knowledge base that allows detailed examination of relationships and causal links within pathways [67]. However, it focuses on humans and well-characterized model species, potentially missing novel or context-specific interactions [68]. Furthermore, as a proprietary tool, IPA can be less accessible and less transparent compared to public databases [68].

miRNA Enrichment Analysis and Annotation (miEAA) facilitates the study of miRNA functions by conducting over-representation analysis (ORA) or gene set enrichment analysis (GSEA) for various functional groups [69]. Tailored for miRNA data, it includes extensive reference sets connecting miRNAs to biological processes. For example, the use of miEAA in autoimmune diseases highlighted dysregulated miRNAs linked to T-cell activation and cytokine signaling in osteoarthritis [70]. The key strengths of miEAA are its ability to perform ORA and GSEA tailored for miRNAs and its extensive annotation database, enhancing functional interpretation [62]. However, its limitations include restricted applicability to well-studied species [96]. Although updating to newer versions has expanded species options, some rare animals are still not included.

4.4 | ML and Integrative Tools

Multimodal Annotation Generated Pathogenic Impact Evaluator (MAGPIE) is an ML-based tool that combines several annotations, such as sequences, conservation, and functional data, to predict the pathogenicity of genetic variants [72]. Introduced in 2024, MAGPIE was trained and validated on numerous pathogenic and benign variants from ClinVar, demonstrating accuracy in interpreting Mendelian disorders by analyzing epigenetic, structural, and evolutionary features of variants [72]. MAGPIE showcased superior predictive power, effectively interpreting rare variants, and can be applied to many types of genomic variant analyses [72]. However, since MAGPIE studies each variant individually, it is less effective at recognizing complex or polygenic diseases [72]. It also fails to integrate patient phenotypes or medical records, which may reduce accuracy when clinical context is needed [72]. Furthermore, MAGPIE performs better on exonic regions than on noncoding areas, and reliance on multiple annotation tools results in slower runtimes, potentially limiting its clinical application [72].

4.5 | Network Visualization and Analysis Tool

Cytoscape is a free, open-source tool for visualizing, drawing, and exploring biological networks, where nodes represent molecules, such as proteins or genes, and lines represent their interactions. The platform enables network arrangement, search for specific elements, overlay of gene-expression or phenotype data, and integration with functional annotation databases [85]. A major advantage is its plug-in system, which supports over 250 add-ons for tasks such as module detection, pathway enrichment, and external databases access [87]. Although Cytoscape's use in FMF research is limited, it presents an opportunity to construct miRNA interaction networks beyond simple differential expression analyses. In RA, Cytoscape has been extensively applied. The molecular complex detection (MCODE) plugin has been used to identify densely connected modules in protein–protein interaction networks, which were then incorporated into competing endogenous RNA (ceRNA) networks linking lncRNAs, miRNAs, and genes [86]. For instance, one RA study constructed a ceRNA network containing two key lncRNAs, 20 miRNAs, and nine genes, while another built a circRNA–miRNA co-expression network with 228 interactions, revealing the involvement of apoptosis, inflammation, and autophagy using the GO and KEGG analysis [31]. Cytoscape's modular design allows customization of network analyses and direct integration of expression or phenotype data onto interaction maps [85, 87]. While modern versions can handle large networks, intensive layouts, and clustering can still overwhelm memory or CPU resources, necessitating simplified visualizations or external tools [88]. Additionally, due to the extensive array of add-ons, newcomers may find the software challenging to master [88].

5 | New Frontiers in FMF Research: Challenges and Future Perspectives

The inclusion of bioinformatics into FMF research holds significant potential. By combining computational analyses with molecular and clinical data, researchers can map regulatory networks driving inflammation and disease variability in patients. Advanced data-driven approaches could help identify potential gene targets and signaling pathways influenced by miRNAs, improving understanding of how dysregulated post-transcriptional control contributes to disease progression. Predictive modeling and multi-omics analyses could enable anticipation of flares, assessment of treatment response, and detection of subtle molecular patterns that conventional methods may miss. Although exploratory, these approaches could transition FMF research toward a more precise, mechanistic, and individualized field.

For greater accuracy, multiple bioinformatics tools are often used sequentially or in combination. For instance, in RA, initial miRNA target predictions using TargetScan are cross-referenced with miRDB, and high-confidence interactions are validated through miRTarBase and reporter assays [38]. Likewise, DIANA-miRPath and IPA are applied for pathway enrichment: DIANA assesses regulatory impacts via KEGG/GO databases, while IPA links affected genes to upstream regulators and canonical pathways. This strategy showed that miRNA expression changes in T-cell signaling and cytokine cascades in

SLE [57]. Recent advancements also involve integrating ML models with transcriptomic data to predict FMF-related outcomes such as flare frequency and drug responsiveness, leveraging miRNA targets from miRTarBase and pathway annotations from IPA [78, 90]. Together, these tools provide a synergistic framework: prediction algorithms refine target selection, databases validate interactions, and functional analysis tools elucidate biological significance.

Despite these advancements, bioinformatics faces key limitations that hinder its use in FMF research. Large, inclusive FMF-specific datasets are scarce, as most studies rely on small cohorts [97]. Variations in patient populations and laboratory processes reduce reproducibility and generalizability [10]. Additionally, computational predictions, such as miRNA targets or pathogenic variants, require experimental validation [98]. Clinical heterogeneity adds another layer of complexity [30], necessitating approaches that can accommodate diverse phenotypes and confounders. Moreover, the overlap of inflammatory pathways with other diseases makes it challenging to distinguish FMF-specific signals from generalized inflammatory noise [99]. Addressing these issues requires careful study design with adequate sample sizes, matched controls, cross-validation, and integration of computational, experimental, and clinical data.

Future FMF research should focus on multi-center data collection, similar to IBD and SLE initiatives [100, 101]. Validation of computational predictions via experimental methods, such as luciferase reporter assays or biomarker testing in patient samples, is essential to translate findings into clinical insights. Integrating multi-omics data can uncover comprehensive molecular signatures in FMF. ML algorithms applied to longitudinal patient data can further support predictive modeling of disease flares and treatment responses, as demonstrated in SLE and RA.

6 | Conclusion

The study of miRNAs in FMF is increasingly becoming a key focus in FMF research. While several bioinformatics tools have contributed to identifying dysregulated miRNAs and potential targets, their integration in FMF remains underdeveloped compared to other inflammatory diseases. Tools like miRDeep2, miRDB, miRTarBase, DIANA-miRPath, IPA, and MAGPIE, though not yet widely applied in FMF, have successfully mapped immune pathways and gene regulation in related conditions and could be adapted for FMF. Strategically combining these tools offers a powerful pipeline for unraveling disease-specific mechanisms. Key challenges include the limited availability of large, high-quality FMF datasets, the complexity of genotype–phenotype correlations, and the need for experimental validation of in silico predictions. Overcoming these limitations will require interdisciplinary data-sharing platforms, integrated multi-omics analyses, and the application of ML. With these advancements, bioinformatics can shift from a supportive role to a central tool in FMF research, facilitating the identification of novel diagnostic markers and enabling more effective, personalized treatment strategies. The future of FMF precision medicine will depend on applying these computational resources to clarify the molecular complexity of the disease.

Author Contributions

Zeinab Skaineh: investigation, writing – original draft preparation (equal). **Razane Hammoud:** investigation, writing – original draft preparation (equal). **Ahlam Chaaban:** conceptualization, review and editing. **Eliana Eldawra:** supervision, review and editing. **José-Noel Ibrahim:** conceptualization, supervision, review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Transparency Statement

The lead author José-Noel Ibrahim affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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