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Review

Somatic mutations in rheumatological diseases: VEXAS syndrome and beyond

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Abstract

Discovery of the VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome demonstrates that somatic mutations in haematological precursor cells can cause adult-onset, complex inflammatory disease. Unlike germline mutations, somatic mutations occur throughout the lifespan, are restricted to specific tissue types, and may play a causal role in non-heritable rheumatological diseases, especially conditions that start in later life. Improvements in sequencing technology have enabled researchers and clinicians to detect somatic mutations in various tissue types, especially blood. Understanding the relationships between cell-specific acquired mutations and inflammation is likely to yield key insights into causal factors that underlie many rheumatological diseases. The objective of this review is to detail how somatic mutations are likely to be relevant to clinicians who care for patients with rheumatological diseases, with particular focus on the pathogenetic mechanisms of the VEXAS syndrome.

Key words: VEXAS syndrome, somatic mutations, clonal haematopoiesis, autoinflammatory disease, autoimmune disease

REVIEW

Rheumatology key messages

- Most genetic studies in rheumatology focus on heritable variants in the germline.
- Somatic mutations are associated with an expanding list of autoimmune and autoinflammatory diseases.
- VEXAS syndrome is an adult-onset, monogenic disease caused by somatic mutations in haematological cells.

Introduction

Discovery of monogenic diseases has traditionally prioritized investigation of early-onset illnesses or cases of familial aggregation of disease. Genetic mutations in the germline define an increasing list of heritable, monogenic diseases that typically manifest early in life. In contrast, emphasis on identifying genetic causes of disease in the germline may be too restrictive for rheumatological diseases that have peak incidence in adulthood, including diseases exclusively restricted to later life, such as GCA. Further, familial aggregation of disease is uncommon for most rheumatological diseases, arguing against Mendelian patterns of inheritance. In contrast to germline mutations, alterations in DNA that occur after the first zygotic division are referred to as somatic mutations. Somatic mutations increasingly occur throughout the lifespan, from early embryogenesis through adulthood. Mutations that are acquired outside of gonadal tissue are not heritable, thus familial aggregation of disease would not be expected under this paradigm. While somatic mutations have a well-established causal role in cancer, the role of somatic mutations in rheumatological disease is less clear.

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To date, most genetic studies in rheumatological diseases have focused upon the effect of germline variants or common disease risk-conferring single nucleotide polymorphisms. While these studies have broadened understanding about the pathogenesis of many diseases, genetic variation in the germline may play less of a role in diseases that emerge later in life. The VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome is caused by somatic mutations in the gene *UBA1* that result in systemic inflammation and progressive bone marrow failure, with initial clinical symptoms manifesting in the fifth decade of life or later [1]. The recent discovery of the VEXAS syndrome proves that somatic mutations can cause exclusively adult-onset, inflammatory, monogenic syndromes.

This review focuses on the role of somatic mutations within the field of rheumatology. Understanding to what extent cell-specific acquired mutations are selected for and expanded within an inflammatory environment *vs* those that directly contribute to inflammation is likely to yield key insights into both disease onset and progression in rheumatological diseases. As sequencing methods continue to improve, the understanding of how somatic mutations evolve throughout the lifespan of healthy individuals and the relationship of tissue-restricted mutations to disease states will expand [2, 3]. The causal mechanism of the VEXAS syndrome will be reviewed. Examples that illustrate how somatic mutations are likely to be relevant to clinicians who care for patients with rheumatological diseases will be highlighted.

Somatic mutations in healthy individuals

Genetic alterations in blood and tissue are frequent and dynamic events linked to cell replication. Exposure to mutagenic environmental and endogenous factors present continuous challenges to the genome. In some cases, mosaicism is physiological, with the most notable examples being V(D)J segment rearrangements that result in the wide diversity of the T cell receptor repertoire [4] or somatic hypermutation in proliferating germinal centre B cells [5]. Genetic analysis of tissues in healthy individuals and in disease states has revealed tissue-restricted mutations that occur across the age spectrum [2, 6]. In an interesting case study, using brain as a control tissue, ~600 somatic variants were found in the blood of a 115-year-old healthy woman [7]. While most acquired mutations likely have a neutral effect on health, a few are deleterious, and in some instances, somatic mutations can be protective, such as revertant mosaicism in genodermatoses [8].

Clonal haematopoiesis of indeterminate potential

Somatic mutations in healthy populations have been best characterized in blood, likely because it is an easily accessible tissue source. In some healthy individuals, mutations in a set of genes associated with myeloid neoplasms have been found in clonal populations of peripheral blood cells [9–11]. Clonal haematopoiesis of indeterminate potential (CHIP) is defined by the detection of somatic mutations in at least one of these genes, typically at a variant allele fraction of $\geq$2% in peripheral blood, in a person without clinical evidence of cytopenia or clonal disorders [12]. The prevalence of CHIP increases with age, and the most common genes associated with CHIP are *DNMT3A*, *TET2*, *JAK2* and *ASXL1* [10, 11, 13] (Table 1). Most recently, CHIP has been associated with an increased risk for cardiovascular disease (CVD) and ischaemic stroke, independent of traditional risk factors such as smoking and dyslipidaemia [11, 14].

Understanding the mechanistic consequences of CHIP will likely be relevant to rheumatologists and immunologists. In mice, loss-of-function of *Dnmt3a* or *Tet2* mediates inflammation by activating the NLRP3 inflammasome and increasing production of key cytokines, including IL-1β and IL-6, and chemokines leading to an increase of monocyte-recruiting P-selectin within the aorta [14–17]. Notably, serum high-sensitivity CRP, which is produced by hepatocytes in response to IL-6 [18], is higher in patients with CHIP [19]. In the Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) trial, targeting IL-1β reduced risk of myocardial infarction and stroke compared with placebo [20, 21]. The favourable response to canakinumab was more pronounced in patients with *TET2* CHIP mutations [22]. Interestingly, patients harbouring large CHIP clones (allele fraction >10%) and the *IL6R* signal attenuating p. Asp358Ala variant were protected against CVD [23]. Collectively, these findings raise questions about the relationship between CHIP and inflammatory diseases, particularly in diseases affecting the vasculature.

CHIP has been studied in RA, ANCA-associated vasculitis (AAV), ulcerative colitis (UC), Schnitzler syndrome (SchS), SSc and GCA [13, 24–27]. Despite years of systemic inflammation, CHIP prevalence was not increased above controls in 59 RA patients, nor did its presence correlate with disease activity [13]. In contrast, in a study of 112 AAV patients, the prevalence of CHIP mutations was found to be significantly elevated above that of age-matched healthy controls. Those with CHIP had less renal and nervous system involvement and ANCA-induced neutrophil reactive oxygen species production [24]. Compared with age-matched controls, CHIP prevalence was significantly elevated in SSc patients younger than 50 years of age, a difference that diminished with ageing, and was associated with a higher proportion of patients with serum anti-RNA polymerase III antibodies [27]. In ulcerative colitis, *DNMT3A* and *PPM1D* CHIP was significantly increased above historical controls. While CHIP status was not linked to differences in serum TNF-α, patients with *DNMT3A* CHIP displayed significantly higher serum IFN-γ levels [25]. In general, these studies were cross-sectional with small sample size, limiting the ability to accurately evaluate the relationship between CHIP mutations and clinical outcomes. Therefore, whether CHIP expansion is driven by inflammation or directly contributes to the pathogenesis of inflammatory conditions remains to be determined.

Table 1 Clonal haematopoiesis of indeterminate potential—most commonly associated genes

	DNMT3A	*TET2*	*ASXL1*	*JAK2*
Gene function	Catalyses cytosine methylation	Begins cytosine demethylation	Chromatin regulator and histone modifier	Component of critical cell signalling pathway
Associated cytokines	Increases expression of CXCL1, CXCL2, IL-6 and CCL5	Increased expression of IL-6 and IL-1β transcripts; elevated serum CXCL9, CCL5, IL-10 and TNFα in serum; CCL2, CCL4, IL-12 p40, RANTES, IL-6 and IL-1β may also be elevated in serum but reports are conflicting; conflicting reports on whether TET2 mutant human cells produce more IL-6 and IL-1β transcripts	No significant associations with cytokine production	Increased transcription of IL-6, IL-1β, TNFα and CCL2
Associated cells	Leads to myeloid skewing	Differentiation skews to myelomonocytic and macrophage lineages; oligo- or monoclonality develops in B cell compartment	Total loss leads to pancytopenia and myeloid skewing; haematopoietic-restricted loss leads to impaired erythroid differentiation and age-dependent decreases in mature B-cells, neutrophils and monocytes; knock-in models show myeloid skewing, increased platelets and fewer erythrocytes with some reports of pancytopenia or leukopenia	Myeloid skewing, increases in erythroblasts and platelet counts
Clinical associations	Systemic mastocytosis, T cell acute lymphoblastic leukaemia, DNMT3A overgrowth syndrome	Essential thrombocythemia, polycythemia vera, primary myelofibrosis, systemic mastocytosis	Bohring–Optiz syndrome, systemic mastocytosis, chronic myelomonocytic leukaemia	Crohn's disease, essential thrombocythemia, polycythemia vera, primary myelofibrosis, Budd–Chiari syndrome
Drugs to target effects of mutation	Hypermethylating agents or high dose anthracyclines	Hypomethylating agents, azacytidine, E3330 (NF-κB and STAT3 inhibitor), SHP099 (SHP2 inhibitor)	BAP1 inhibition (no clinically available drugs), PUGNAc, virinostat	Ruxolitinib, tofacitinib, baricitinib, fedratinib, givinostat

BRCA1-associated protein-1; CCL: chemokine (C-C motif) ligand; CXCL: chemokine (C-X-C motif) ligand; NF-κB: nuclear factor-κB; RANTES: regulated on activation, normal T cell expressed and secreted; SHP2: Src homology-2 containing protein tyrosine phosphatase-2; STAT3: signal transducer and activator of transcription-3.

Examples of somatic mutations in rheumatological diseases

Over the past 40 years, the identification of genetic variation contributing to inflammatory disease has mostly focused on variation transmitted through the germline. Goodnow proposed a pathogenic mechanism similar to the development of malignancy: dictated by basal mutational rate and mutation type, an accumulation of somatic 'hits' in genes designed to uphold self-tolerance could ultimately lead to autoimmunity [28]. Indeed, a cascade of sequentially acquired somatic mutations within B lymphocytes has been recently reported, starting with a V(D)J recombination causing benign RF formation and ultimately leading to this B cell clone producing pathological RF driven cryoglobulinemic vasculitis [29]. However,

the possibility of somatic mosaicism as a causal mechanism of inflammatory disease was further raised by the existence of mutation-negative patients that closely phenocopied established autosomal dominant monogenic diseases. The following narrative is not meant to be an exhaustive discussion of all somatically driven inflammatory diseases, but rather to illustrate a handful of clinically relevant concepts underlying somatic mosaicism. A list of rheumatological diseases associated with somatic mutations is presented in Table 2.

Somatic mutations phenocopy germline mutations in inflammatory diseases

Autoimmune lymphoproliferative syndrome (ALPS) is a prototypical autoimmune disorder that is caused by both germline and somatic mutations [30]. Its inheritance is autosomal dominant with clinical features of persistent lymphadenopathy and splenomegaly secondary to abnormal lymphoid proliferation, including the hallmark increased circulating $CD4^{-}CD8^{-}$ (double-negative) $TCR\alpha/\beta^{+}$ T lymphocytes, hypergammaglobulaemia, and evidence of autoantibodies and autoimmunity (mainly haemolytic anaemia, idiopathic thrombocytopenia and neutropenia) [44]. While germline heterozygous dominant negative mutations within proapoptotic *TNFRSF6*, encoding Fas/CD95, have been identified in about 65% of ALPS cases [45–47], close disease phenocopies have been found to have mutations in other proapoptotic genes, such as caspases 8 or 10 [48, 49], *FasL* [50], *KRAS* [31, 32] and *NRAS* [33].

Illustrating an example of a somatic mutation phenocopying its associated germline disease, focusing on germline *TNFRSF6* mutation negative ALPS patients with no family history of disease, somatic *TNFRSF6* mutations were first identified in fluorescence-sorted peripheral double-negative T cells [30]. Depending on the mutation, ALPS also displays incomplete clinical penetrance, with some family members remaining completely asymptomatic [51]. To help explain intrafamilial heterogeneity, a somatic 'second hit' model was proposed similar to malignancy and studied via genetic comparison of symptomatic ALPS patients with their older asymptomatic relatives. Sequencing DNA from sorted double-negative T cells and various other cell types revealed additional somatically acquired genetic perturbations in the other *TNFRSF6* allele, perhaps causing an earlier onset of an otherwise low penetrance mutation [52]. Given that ALPS patients have a significantly higher risk for developing multiple different types of malignancies after years of disease [53], it is likely that the loss of normal apoptosis also leads to additional somatic mutational burden over time.

Somatic mutation detection method depends on mutational burden

Previously named cryopyrin-associated periodic fever syndrome (CAPS) [54], NLRP3-associatiated autoinflammatory disease (NLRP3-AID) encompasses a group of familial and sporadic autosomal dominant diseases characterized by gain-of-function mutations in *NLRP3*, leading to increased inflammasome activity. Their phenotypic presentation is broad [55], leading to a continuous spectrum of diseases previously named familial cold autoinflammatory syndrome (FCAS), Muckle–Wells syndrome (MWS) and neonatal-onset multisystem inflammatory disorder (NOMID) or chronic infantile neurological, cutaneous and articular (CINCA) syndrome. Given their milder phenotypes, FCAS and MWS tend to be familial [56], while

Table 2 Inflammatory diseases associated with somatic mutations

Disease	Gene	Chr.	Mechanism	Reference
Autoimmunity				
ALPS	*FAS*	Chr10	LOF	[30]
RALD	*KRAS*	Chr12	GOF	[31, 32]
	NRAS	Chr1	GOF	[33]
Felty syndrome	*STAT3*	Chr17	GOF	[34]
Autoinflammatory				
NLRP3-AID	*NLRP3*	Chr1	GOF	[35]
AIFEC	*NLRC4*	Chr2	GOF	[36]
TRAPS	*TNFRSF1A*	Chr12	GOF	[37]
Blau syndrome	*NOD2*	Chr16	GOF	[38]
SAVI	*TMEM173*	Chr5	GOF	[39]
VEXAS	*UBA1*	X	LOF	[1]
JAK1 GOF	*JAK1*	Chr1	GOF	[40]
MDS–Behçet's	N/A	Chr8	Trisomy	[41, 42]
ECD	*BRAF*	Chr7	GOF	[43]

AIFEC: autoinflammation with infantile enterocolitis; ALPS: autoimmune lymphoproliferative syndrome; ECD: Erdheim-Chester disease; GOF: gain-of-function; LOF: loss-of-function; MDS: myelodysplastic syndrome; NLRP3-AID: NLRP3-associated inflammatory disease; RALD: RAS-associated autoimmune leukoproliferative disease; TRAPS: tumour necrosis factor receptor-associated periodic syndrome; SAVI: STING-associated vasculopathy with onset in infancy; VEXAS: vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic.

the more severe NOMID is mostly sporadic [57]. Whereas some *NLRP3* mutations are closely linked to disease severity, some are associated with more heterogeneous phenotypic presentations [58, 59], possibly due to other genetic or environmental factors.

Sporadic NLRP3-AID may be due to *de novo* germline or acquired somatic mutations. Considering that about 40% of NOMID patients are negative for germline *NLRP3* mutations [57, 58, 60, 61], focus turned to investigation of possible somatic mutations. Early efforts [60] failed to identify mosaicism within 14 *NLRP3* mutation-negative NOMID patients using traditional bidirectional Sanger sequencing, which has a lower limit of detection of around 20% somaticism [62]. Other groups have utilized more sensitive approaches to detect mosaicism. Applying knowledge of the pathophysiology of this disease, Saito *et al.* demonstrated enrichment of mutation-positive cells from a mixed population by sequencing cells undergoing LPS-induced cell death [63]. A large international effort employed subcloning of amplicons followed by Sanger sequencing and identified a mosaicism allele frequency of 4.2–35.8% in about 70% of *NLRP3* mutation-negative NOMID patients, highlighting that ~30% of NOMID cases are due to mosaicism [61]. Given the laborious and time-consuming process of subcloning, next-generation sequencing (NGS) should revolutionize the hunt for disease-causing mosaicism [64]. NGS can detect NLRP3 mosaicism as low as 2% [65]. However, with increased read coverage, detecting an allele frequency of about 1% or lower can be achieved [35]. In practice, these studies illustrate that outside of labour-intensive subcloning or cell sorting, conventional Sanger sequencing is usually not sensitive enough to reliably detect the presence of low-level somaticism and NGS has revolutionized the ability to rapidly detect such mutations.

A recent study examined 223 unrelated patients harbouring either somatic or germline *NLRP3* mutations and found a locational segregation between these two groups, with only a small overlap [66]. The most common amino acid change in mosaic patients, p. Glu569, has only been reported once as a germline mutation in a severe case of NOMID, causing the authors to speculate that somatic mutations in *NLRP3* would likely be incompatible with life if found in the germline state, and conversely, known germline *NLRP3* mutations would likely cause mild or subclinical disease if somatic. Finally, a correlation between phenotypic severity and level of mosaicism was not identified.

Myeloid restricted somatic mutations may cause late-onset autoinflammatory disease

Rowczenio *et al.* presented a series of eight patients with late-onset periodic urticaria, fevers and progressive bilateral sensorineural hearing loss, consistent with MWS. Amplicon-based NGS done at mean coverage 3500× revealed *NLPR3* mosaicism in all cases, where the mutational burden was highest in the myeloid cells [67]. Interestingly, they also reported a patient with symptom onset at age 46 and progressive disease displaying an allele frequency increasing from 5.1 to 45% over the span of 12 years. While *NLRP3* somatic mutations have mostly been demonstrated in paediatric patients [61, 63, 65, 68–71], these mutations can also cause adult-onset disease [67, 72–74]. Two reports described patients in their 50 s and 60 s with symptoms consistent with MWS, both found to have low-level mosaicism mostly confined to their myeloid cells [72, 73]. The commonality among mosaic NLRP3-AID is the variant allele frequency being highest within the myeloid lineage, indicating a survival benefit, an indirectly related expansion of the myeloid compartment due to normal aging, or both [67].

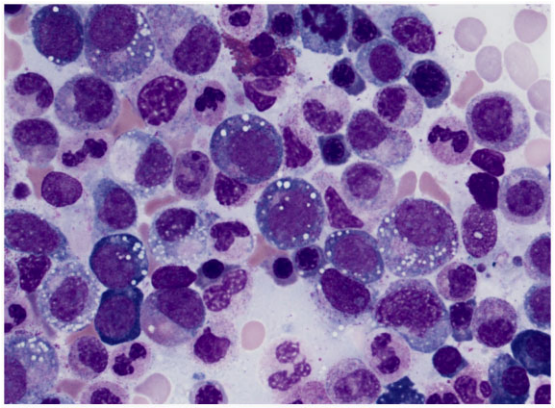
Somatic mutations link inflammation and malignancy

The inflammasome-driven acquired autoinflammatory condition Schnitzler syndrome (SchS) may be considered a phenocopy of late-onset NLRP3-AID mosaicism, but SchS is usually distinguished via the presence of serum monoclonal IgMκ gammopathy [75]. Given the similarity of these conditions, efforts were made to identify *NLRP3* mosaicism within SchS. However, genetic interrogations of 21 patients with classical SchS failed to identify a clear disease-causing germline or somatic mutations in *NLRP3* [76]. Given that around 20% of SchS patients progress to clinically overt lymphoproliferative disorders, including Waldenstrom's macroglobulinaemia (WM) [77], and that 90% of WM carry the *MYD88* L265P gain-of-function (GoF) mutation [78], it was postulated that *MYD88* GoF could underlie the pathogenesis of SchS [76]. Indeed, Pathak *et al.* identified *MYD88* L265P in the peripheral blood of 9 of 30 patients using allele-specific oligonucleotide PCR with a lower limit of detection of 1% allele frequency [26], while excluding NLRP3 mosaicism. Predating this important finding, *MYD88* GoF mutations had been identified as somatic variants in various blood malignancies [78–80], as well as a germline mutation patient with early-onset severe, episodic arthritis and rash [81], illustrating a link between autoinflammatory disease and malignancy.

Discovery of the VEXAS syndrome

The VEXAS syndrome is a newly described monogenic disease of adulthood caused by somatic mutations in *UBA1* [1]. This syndrome was identified using a genotype-first approach. WES data from 2560 individuals were screened for novel, shared, genetic variants in 841 genes related to protein ubiquitylation, which initially identified three individuals with missense mutations in codon 41 of *UBA1*. These individuals were older men with treatment-refractory severe inflammatory disease and progressive bone marrow failure. Detailed examination of bone marrow aspirates demonstrated characteristic cytoplasmic vacuoles in the myeloid and erythroid precursor cells (Fig. 1). Subsequent, clinical assessment for patients with severe multisystem inflammatory disease and progressive bone marrow failure led to the

Fig. 1 Bone marrow aspirate from patient with the VEXAS syndrome



Characteristic vacuoles are present in myeloid and erythroid precursor cells. Vacuoles are sensitive for the VEXAS syndrome but are not specific for the disease. Presence of vacuoles likely reflects defects in cytoplasmic ubiquitylation related to somatic mutations in *UBA1*, the master switch of the ubiquitin pathway. Image courtesy of Dr Katherine Calvo, National Institutes of Health.

identification of 25 adult patients with novel missense mutations in *UBA1*, described in the initial report of the VEXAS syndrome [1].

Several reports on the VEXAS syndrome from cohorts around the world have confirmed the stereotypical nature of the disease. Common inflammatory features include fever, chondritis, vasculitis, neutrophilic dermatosis and sterile alveolitis. Given this particular collection of systemic inflammatory features, patients with VEXAS syndrome are often clinically diagnosed with several rheumatological conditions, including relapsing polychondritis, Sweet syndrome, adult-onset Still's disease, polyarteritis nodosa and GCA. The VEXAS syndrome is also a haematological disease, with macrocytosis being an early manifestation in almost all patients. Patients often develop cytopenias and may eventually require blood and platelet transfusions. In fact, a clinical algorithm based on male sex and mean corpuscular volume >100 and/or platelet count <200 identified patients with the VEXAS syndrome within a cohort of patients clinically diagnosed with relapsing polychondritis with near-perfect accuracy [82]. Serial bone marrow biopsies demonstrate evolving myelodysplastic syndrome or multiple myeloma. To date, all patients with the VEXAS syndrome have vacuoles in myeloid and erythroid precursor cells on bone marrow aspirates—a finding that is sensitive for the diagnosis but is not pathognomonic since similar vacuoles have been described in association with neoplasms, copper deficiency, alcoholism and other states of malnutrition [83].

Conjugation of ubiquitin to protein substrates marks them for degradation or functional modulation. Cellular ubiquitin tagging employs an enzymatic cascade, ultimately leading to ubiquitin becoming covalently bound to its target protein. In this process, the E1 enzyme activates and transfers ubiquitin to ~40 different E2 ubiquitin ligases, which in turn deliver it to target proteins with the cooperation of over 600 substrate-specific E3 ubiquitin ligases [84]. *UBA1* (formerly known as *UBE1*) encodes the canonical mammalian E1-ubiquitin ligase and exists as two isoforms in the physiological state: a nuclear form initiated at p.M1 and a cytoplasmic form initiated at p.M41 [85, 86]. Almost all VEXAS mutations occur at p.41M, abolishing this alternative start site. Surprisingly, immunohistochemistry can still identify UBA1 within the cytoplasm of patient monocytes, which led to the hypothesis of an even further downstream start codon being used. Indeed, a smaller isoform, corresponding to start site p.M67 can be translated in cells from patients with VEXAS, despite this isoform showing impaired ubiquitin thioester formation. Interestingly, certain mutations within UBA1 had previously been shown to cause temperature-sensitive impairment of its enzymatic activity in several murine cell lines [87–89]. Outside of the p.M41 variant, p.Ser56Phe has been described in one patient and decreases ubiquitin thioester formation at 37°C, but not at 4°C. Another novel mutation at a splice acceptor site (c.118-1G>C) is believed to cause aberrant RNA splice products [90]. Collectively, all VEXAS mutations described to date cause loss-of-function of normal UBA1 and occur exclusively in males, except for females with concomitant monosomy X [91–94].

The VEXAS phenotype is one of extreme inflammation, characterized by significantly elevated CRP, an interferon-induced protein signature, and increased serum neutrophil attracting IL-8. A cellular impaired ubiquitin-proteasome system leads to an accumulation of proteins, triggering an unfolded protein response as shown by increased eIF2-α phosphorylation and XBP1 splicing in patient monocytes. Given that ubiquitylation is a key regulator of the immune response [95], multiple mechanisms likely contribute to this complex inflammatory phenotype. This complexity is supported by the relative resistance to nearly all target-specific anti-rheumatic agents, while systemic glucocorticoids display the best efficacy. Given significantly elevated CRP levels and increased *IL6* transcripts seen in VEXAS $CD14^+$ monocytes, tocilizumab does ameliorate some manifestations, yet elevations of serum proinflammatory cytokines persist and it does not alter the progression of disease [96, 97]. It is conceivable, given the pervasive effects of UBA1 dysfunction, more general chemotherapies, such as the hypomethylating agent azacytidine [96] may be more beneficial than targeted therapies. JAKinibs may also be clinically effective [96]. Ultimately, bone marrow transplantation may be curative [92].

VEXAS represents a new class of conditions where somatic mutations in blood cause adult-onset inflammatory diseases with complex phenotypes that do not phenocopy established germline disease. In the context of newly discovered rheumatological diseases, the pathogenetic mechanisms underlying the VEXAS syndrome are particularly novel and compelling. Pathogenic

somatic mutations acquired very early in life usually affect multiple embryonic germ layers and lead to severe, early-onset disease, as illustrated by diseases such as Sturge–Weber syndrome, Proteus syndrome, or McCune–Albright syndrome [98]. In contrast, pathogenic mutations acquired later in life usually display a restriction in affected cell populations, thus are more challenging to detect, and result in less pervasive phenotypes. The VEXAS syndrome, however, does not support this oversimplified view, because this disease can be readily detected in peripheral blood, is associated with a high burden of mutant cells, and leads to a severe clinical phenotype with onset later in life. Although VEXAS is fundamentally a disease of haematopoietic progenitor cells, mutations can be readily detected in peripheral blood due to profound clonal expansion of mutant, circulating myeloid cells, but not T or B lymphocytes, possibly due to negative selection of mutant lymphocytes within bone marrow. A high clonal burden of mutation is almost always observed at time of genetic diagnosis, and preliminary data do not show a correlation between variant allele fraction and disease duration or phenotypic severity [82]. While the exact timing of genetic onset of disease is unknown, somatic mutations in *UBA1* are likely acquired later in life in patients with the VEXAS syndrome, aligning with the observation that clinical symptoms first begin in the fifth decade of life or later. The mechanisms that drive clonal expansion and the rate of expansion are currently unknown.

VEXAS joins an expanding list of monogenic inflammatory diseases caused by genetic perturbations of the ubiquitin–protease system [39, 99]. Discovery of the VEXAS syndrome will likely facilitate identification of additional rheumatological diseases caused by somatic mutations. If somatic mutations occur in blood, patients may present with an overlap of inflammatory and haematological manifestations. A clinical association between autoimmune diseases and blood dysplasia has long been established [100, 101]. Early studies screening for *UBA1* mutations in these kinds of patients demonstrate that VEXAS likely explains many, but not all, of these clinical associations. Genomic interrogation for somatic mutations in genes beyond *UBA1* is warranted in patients with systemic inflammation and conditions like myelodysplastic syndrome or chronic myelomonocytic leukaemia. Additionally, disease-associated somatic mutations may be restricted to specific tissue types in solid organs rather than peripheral blood and thus could be more challenging to identify in absence of biopsy material.

Haematoinflammatory diseases

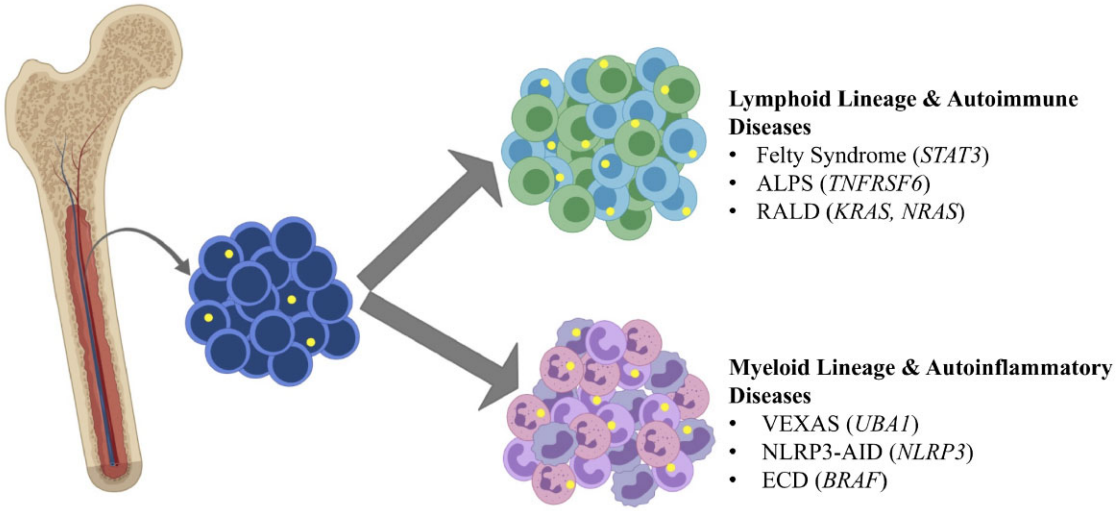
The VEXAS syndrome is a prototype for a new class of diseases. We have proposed the term *haematoinflammatory disease* to define these conditions with the following requirements: (i) somatic mutations in blood cells; (ii) systemic inflammation; and (iii) progression towards myeloproliferative, myelodysplastic or lymphoproliferative diseases [102]. In addition to VEXAS syndrome, a few other established diseases meet this definition.

Erdheim–Chester disease (ECD) is a non-Langerhans-cell histiocytosis with systemic inflammatory features involving cortical bone, skin, lung, aorta, central nervous system and the retroperitoneum [103]. Somatic mutations in *BRAF* within histiocytes are causal in ~50% of patients with ECD, and these patients may benefit from treatment with vemurafenib, a targeted agent to the BRAF protein [43, 104]. Over time, these patients can develop myeloid neoplasia [105]. These observations highlight the principle that somatic mutations can cause systemic inflammatory diseases, inform targeted treatment approaches and carry prognostic information about disease progression.

The VEXAS syndrome and ECD are disease examples of somatic mutations in blood restricted to myeloid lineages, but examples exist of somatic mutations within lymphoid lineages associated with rheumatological diseases (Fig. 2). Felty syndrome is defined by neutropenia, splenomegaly and RA. Somatic *STAT3* mutations in $CD8^+$ T cells have been described in 43% of patients with Felty syndrome [34]. These same mutations have been characterized at similar frequencies in association with large granular lymphocyte leukaemia [106]. Of note, identification of acquired mutations restricted to lymphocytes may be more challenging to detect due to relatively low abundance of these cells in peripheral blood.

In up to half of patients, an autoimmune or inflammatory phenomenon may precede, occur simultaneously with, or follow the development of myelodysplastic syndrome (MDS) [107–109]. Trisomy 8 mosaicism (T8m) has a highly variable phenotype, depending on cells harbouring an extra chromosome 8, including skeletal defects, decreased cognitive ability and dysmorphic features. It may also be associated with haematological and neoplastic disorders, including MDS, in about 10–15% of cases [110, 111]. Behçet's disease (BD) is a systemic autoinflammatory disease defined by recurrent aphthous stomatitis, genital ulcers, intestinal inflammation, rash, non-granulomatous uveitis and thrombosis [112], but can also be rarely complicated by MDS and interestingly, most of these patients have T8m [41]. Supporting that this syndrome may be an entity distinct from classical BD, T8m patients have mostly oral/genital ulcers, rash, fever and recalcitrant intestinal inflammation, while eye lesions are uncommon [42, 113, 114]. While trisomy 8 is insufficient to cause this phenotype, as it is commonly detected in MDS patients [115], its contribution to the BD phenotype remains unclear and is likely influenced by other acquired cytogenetic abnormalities. The role of somatic mosaicism in MDS-associated inflammatory phenomena remains an active field of investigation and could serve to illuminate the underling pathogenesis of more common immune mediated diseases.

Fig. 2 Somatic mutations in bone marrow become lineage restricted to myeloid or lymphoid cell populations



Somatic mutations in bone marrow (yellow circles) can become lineage restricted to lymphoid cells in various autoimmune diseases and to myeloid cells in a range of autoinflammatory diseases. Causative genes are in parentheses and italics. ALPS: autoimmune lymphoproliferative syndrome; ECD: Erdheim–Chester disease; NLRP3-AID: NLRP3-associated inflammatory disease; RALD: RAS-associated autoimmune leukoproliferative disease; VEXAS: vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic. Created with BioRender.com.

Conclusion

Somatic mutations have been associated with an expanding range of rheumatological diseases, and knowledge about these mutations will likely be useful to clinicians in a variety of ways. Identification of somatic mutations and monitoring clonal burden over time has the potential to define new disease syndromes, novel biomarkers of disease activity, inform understanding of pathophysiology and disease prognosis, and unlock novel therapeutic approaches. Discovery of the VEXAS syndrome demonstrates the potential of considering somatic mutations as a causal mechanism of adult-onset complex inflammatory syndromes. Future efforts to identify and characterize these mutations in blood and solid organs will help in understanding the dynamic interplay between inflammation and genomic stability.

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Data availability statement

Data are available upon reasonable request by any qualified researchers who engage in rigorous, independent scientific research, and will be provided following review and approval of a research proposal and Statistical Analysis Plan (SAP) and execution of a Data Sharing Agreement (DSA). All data relevant to the study are included in the article.

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