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Genetics of somatic auto-inflammatory disorders

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Abstract

Systemic autoinflammatory disorders (SAIDs) encompass a heterogeneous group of monogenic disorders characterised by recurrent episodes of systemic and organ-specific inflammation. Genetic studies have facilitated the identification of Mendelian forms of SAIDs but many patients still remain without a diagnosis. Recent studies have uncovered that somatic (acquired) mutations can cause later-onset SAIDs. In this review, we will discuss the current knowledge surrounding the genetics of these acquired auto-inflammatory disorders (AAIDs), with a focus on VEXAS, NLRP3-associated AAIDs and Schnitzler's syndrome and provide suggestions for future research in this field.

Keywords: VEXAS, UBA1, NLRP3, Auto-Inflammatory Disease, SAID, Schnitzler's syndrome.

Introduction

Systemic autoinflammatory disorders (SAIDs), originally defined in 1999, encompass a heterogeneous group of monogenic disorders characterised by recurrent episodes of systemic and organ-specific inflammation (1). Genetic studies have facilitated the identification of Mendelian forms of SAIDs and enabled their stratification. This has provided a greater understanding of the underlying disease mechanisms of inherited SAIDs, and the most promising treatments for them.

As with most Mendelian disorders, the majority of research to date has focussed on the identification of germline inherited mutations as a cause of SAIDs. These mutations are inherited from one, or both, parents and are present in most, if not all, cells of the offspring. More recent studies though, have begun to investigate the effect of somatic (acquired) mutations on SAIDs. While somatic mutations have long been known to play a role in senescence and tumorigenesis, the role of somatic mutations in non-cancer cells is still in its infancy (2, 3). Somatic mutations are those not inherited from a parent, but instead are introduced during the lifetime of the individual as a result of a DNA replication defect or environmental insult (e.g. UV radiation). Any daughter cells that are produced from this cell therefore inherit the mutation, which continues in to subsequent generations. While most somatic mutations are not thought to result in a phenotype, a small number do impact on the cellular biology, with those conferring a selective advantage, known as driver mutations, becoming dominant over non-mutated cells (4). Any phenotype resulting from a somatic mutation therefore depends on the gene it has arisen in as well as what tissues or cell types contain the mutation.

Despite the progress made in identifying novel genetic causes of SAIDs, many patients still remain without a diagnosis. While many of these will be due to germline mutations in genes not currently associated with SAIDs, recent studies have uncovered somatic mutations as a cause of later-onset auto-inflammatory disorders. In this review, we will discuss the current knowledge surrounding the genetics of these acquired auto-inflammatory disorders (AAIDs), with a focus on VEXAS, NLRP3-associated AAIDs and Schnitzler's syndrome.

VEXAS syndrome

VEXAS (Vacuoles, E1-ligase, X-linked, Auto-inflammatory, Somatic) is a recently identified syndrome characterised by systemic inflammation and haematological abnormalities. More detail regarding the clinical phenotype of VEXAS, and the overlap with other haematological and inflammatory disorders, are described elsewhere in this series of reviews.

To date, mutations at just three key sites all clustered around exon 3 of *UBA1*, encoding the major E1 ubiquitin ligase, account for all known VEXAS cases, with the majority being substitution of Methionine-41 (p.Met41) (Figure 1A). Below we give a brief overview of all known mutations published to date.

p.Met41 substitutions

In contrast to the phenotype-first approach taken by most studies, VEXAS was discovered by Beck et al. who undertook a large genotype-first screen of exomes and genomes from 2,560 individuals with undiagnosed recurrent fevers, or an atypical undiagnosed disorder (identified through the Undiagnosed Diseases Program). This screen identified 3 male individuals who all had novel variants in *UBA1* (5). The seemingly heterozygous variants all affected the p.Met41 residue and were absent from public databases, including gnomAD. As *UBA1* is located on the

X-chromosome, and the patients were all male, it was noted that the mutations were in fact acquired (i.e. somatic) variants, and not germline. A further 22 male individuals, all with somatic mutations affecting p.Met41 were subsequently identified, all with a late-onset undiagnosed auto-inflammatory disorder, which the authors called VEXAS. A number of follow-up studies have identified further cases with somatic p.Met41 substitution mutations (6-23). Analysis of all available published reports of VEXAS mutations (to 8th July 2021) reveals the p.Met41 mutations account for over 92% of all VEXAS cases (Supplementary Table 1). By far the most common mutation identified is the c.122T>C, pMet41Thr substitution, which has been found to be the cause of disease in approximately 51% of all known published VEXAS cases (Figure 1B). Two additional variants have also been identified that result in substitution of p.Met41, c.121A>G, p.Met41Val and c.121A>C, p.Met41Leu, causing approximately 21% of VEXAS cases each. All three mutation share a similar disease mechanism, having been found to affect initiation of transcription of the cytoplasmic isoform of UBA1 (also known as UBA1b), which uses p.Met41 as its start codon (5). Instead, an alternative initiation codon is used at p.Met67 resulting in a novel isoform, termed UBA1c, which has reduced function. To date, no studies have yet confirmed whether loss of UBA1b or gain of UBA1c is the main driver of the disease phenotype. In either case, the mutation leads to a global loss of ubiquitination and activation of innate immune pathways and the unfolded protein response (5).

Splice acceptor mutations

The next most common location for VEXAS mutations is the canonical splice acceptor site of *UBA1* exon 3. Mutations affecting either of the immediate 2 base pairs preceding the exon (c.118-1 and c.118-2), or deletion of 8bp in the splice acceptor site (c.118-9_118-2del) account for 6% of known cases (8, 16, 24). As with the p.Met41 substitution mutations, mutation of the

splice acceptor site leads to a reduction of cytoplasmic UBA1. A number of studies have shown the mutations alter the location of splicing at the start of the exon (16, 24). Interestingly, Met41 is the second amino acid encoded by exon 3, and therefore a small shift in the location of the splice site is likely to result in removal of Met41 from the transcript and loss of UBA1b, mimicking the effect of the Met41 substitution mutations.

Ser56Phe mutation

In addition to p.Met41 mutations, just one other mutation has been identified at an alternative amino acid residue. By screening a cohort of cases matching the VEXAS phenotype, Poulter et al. identified a c.167C>T mutation which substituted p.Ser56 for phenylalanine (16). While at a different residue, p.Ser56 and p.Met41 are both encoded by codons in *UBA1* exon 3. In contrast to the p.Met-41 and splice acceptor mutations, however, the p.Ser56Phe mutation did not affect expression of UBA1b, nor did it lead to the formation of the UBA1c isoform, but instead affected the catalytic activity of UBA1, resulting in a temperature dependent reduction in ubiquitination (16). Further studies are required to better understand how the p.Ser56Phe mutation causes VEXAS, with similar clinical manifestations to other VEXAS patients, yet appears to affect both UBA1a and UBA1b isoforms and results in a different disease mechanism than p.Met41 and splice acceptor mutations.

Contribution of *UBA1* mutations to the VEXAS phenotype

UBA1 encodes the major E1 ligase required for the initiation of ubiquitination in most human cells. Furthermore, missense mutations between amino acids 539 and 577 have been associated with an X-linked spinal muscular atrophy (SMA)(25). Given the importance of ubiquitination to cellular homeostasis and the lack of an SMA phenotype in VEXAS patients, it is surprising that mutation of UBA1 results in a late-onset auto-inflammatory disorder. However, this can

be partially explained by this being a somatic disorder, resulting in mutations that are restricted to particular cell types. In contrast to other disorders cause by somatic mutations, for example cancers, where the somatic mutation is a driver mutation, causing a survival advantage in all cell types and daughter cell populations having a higher variant allele frequency (VAF) than parental cells, in VEXAS the effect of the wild-type and mutant alleles appears to differ in different cell types (Figure 1C). This is irrespective of the VAF in the parental progenitor cell. This finding is most striking in B-cells and T-cells, which are almost exclusively wild-type alleles despite originating from lymphoid progenitors which have the mutation as the predominant (>75%) allele (5). In contrast, the majority of neutrophils and monocytes (VAF > 80%) and Megakaryocytes (VAF>90%) are mutation containing cells, deriving from progenitors with VAFs of 80% (myeloid progenitors) and megakaryocyte-erythrocyte progenitors (>65%) respectively (5, 16). Sanger sequencing and digital droplet-PCR of patient cheek buccal cells and fibroblasts shows no evidence of the mutation, indicating the somatic mutation is acquired specifically in multipotent progenitors of the haematopoietic lineage or, like lymphocyte derived cells, the mutation is incompatible with cell survival resulting in only wild-type cells being derived. Although these studies were initially performed on cells separated from peripheral blood of p.Met41 substitution patients, the same cellular distribution of variants has been observed in p.Ser56Phe and splice acceptor mutations (16). To date, no explanation has been given as to why myeloid progenitors favour the mutant allele over the wild-type and conversely how lymphoid lineages are able to exclusively select against the mutant allele in favour of the wild-type allele, given that the haematopoietic stem cells, which they both derive from, harbour both alleles. The exclusivity of choice argues against random selection, suggesting a mechanism must be in place to specifically select mutation or wild-type containing haematopoetic stem cells during the differentiation process, however this mechanism is not yet understood.

As well as the specific cell types with the mutation affecting the phenotype, the proportion of mutation-containing alleles compared to the wild-type allele is also likely to have some influence. To date, most cases of VEXAS have the mutation as the predominant allele in peripheral blood compared to the wild-type allele. This same predominance is also observed in DNA extracted from bone marrow biopsy tissue. It is not yet known, however, at what frequency the mutant allele is when the VEXAS phenotypes develop, nor what the initial clinical manifestations are. A number of studies have investigated the change in allele frequency over time, in some cases going back 5-10 years using stored biopsy derived DNA, however in each case there was little change over time, with the mutant allele being predominant (16). This suggests that in the early stages of clinical presentation of VEXAS, the mutation is already predominant in the cell types it is in. It is likely to be for this reason that stem cell transplantation remains the most effective treatment for VEXAS.

Atypical VEXAS cases

Since the initial finding of UBA1 p.Met41 substitution mutations as a cause of VEXAS, genetic screening using a phenotype-first approach has identified approximately 100 cases with a typical VEXAS phenotype. This has resulted in an expansion of the frequency and spectrum of mutations, with the identification of a further 2 hotspot regions within *UBA1* exon 3. Interestingly, a number of atypical cases have also been identified which is gleaning new insights into this disease. For example, while the majority of published cases are male, a small number of female cases have also been identified. While this finding was unexpected, due to females having a second non-mutated X chromosome, these cases can be explained either by monosomy X (6, 7) or by the extremely low allele frequency at which the mutation was found (0.14%) (22). It therefore still remains unclear whether VEXAS can indeed occur in females

with a normal bone marrow karyotype. Despite the rare occurrence of these cases, a differential diagnosis of VEXAS should not be ruled out in females with an undiagnosed AAID, particular in those with vacuolated bone marrow aspirate. In the coming years, as more cases are identified with somatic mutation of *UBA1,* it is likely that more atypical VEXAS cases will be identified.

UBA1 mutation-negative VEXAS cases

Through sequencing of cases with a VEXAS-like phenotype, unsolved cases without mutations in *UBA1* have also been identified. One possibility is that these cases have mutations elsewhere in *UBA1*, outside of the residues/exons screened, as many have only been sequenced at the known mutation sites. Given the disease mechanisms is alteration or loss of p.Met41, leading to loss of the UBA1b isoform, the probability of finding mutations away from p.Met41 is small. The identification of the p.Ser56Phe mutation, however, has opened up the possibility that similar substitution mutations may exist in rare cases, that affect UBA1 catalytic activity rather than UBA1b transcription. A second possibility is that these cases don't have VEXAS and therefore further investigation of their phenotype is required. A third possibility is that the unsolved cases have VEXAS due to a currently unidentified VEXAS disease gene. The knowledge gained from the identification of *UBA1* mutations would suggest that a second gene in the ubiquitination pathway, such as an E2 ligase enzyme, may also cause a similar phenotype. Sequencing of well phenotyped, *UBA1*- mutation negative, VEXAS cases could therefore identify novel disease gene(s) and should be a priority for future research.

Acquired NLRP3-associated autoinflammatory disease (NLRP3-AID)

NLRP3 stands for NACHT (NAIP, CIITA, HET-E, and TP1), LRR (leucin rich repeat) and PYD (pyrin) domain-containing protein 3. It is encoded by the *NLRP3* (formerly known as

cryopyrin) gene on chromosome 1q44. NLRP3 functions as an intracellular sensor for the detection of damage-associated or pathogen-associated molecular patterns (DAMP or PAMP, respectively)(26). Activation of NLRP3 results in the assembly of the multimeric complex known as the NLRP3 inflammasome. The PYD domain of NLRP3 recruits an adaptor molecule called apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), via a homotypic interaction with its PYD domain. Following this, pro-caspase-1 is recruited to ASC via a homotypic interaction of CARD domains, leading to caspase-1 activation. There is subsequent caspase-1 mediated processing of the potent pro-inflammatory cytokines IL-1β and IL-18 from their inactive precursor states to their active forms. In addition, caspase-1 cleaves gasdermin D, whose N-terminal cleavage product forms pores in the cell membrane causing pyroptosis, a type of inflammatory cell death.

Inappropriate activation of inflammasomes can lead to diseases collectively known as inflammasomopathies (27). A spectrum of inflammatory conditions previously known as cryopyrin-associated periodic syndrome (CAPS) and now termed NLRP3-AID, have been linked with heterozygous gain-of-function (GoF) variants in *NLRP3 (28).* The GoF mutations in *NLRP3* decrease the threshold for activation of the NLRP3-inflammasome (29). The mildest form of NLRP3-AID is known as familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) is of moderate severity, whilst neonatal-onset multisystem inflammatory disease (NOMID; also known as CINCA) is the most severe of three disease entities. NLRP3-AIDs associated with germline *NLRP3* mutations (inherited or sporadic) tend to present in the neonatal period, childhood or early adulthood, with first presentation in late adulthood being extremely rare. Symptoms such as fever, urticarial-like non-pruritic rash [termed neutrophilic urticarial dermatosis (30)] and joint involvement are shared by all forms of NLRP3-AID. However, short-lasting episodic fevers in FCAS lasting 1-2 days are typically

triggered by exposure to the cold. In patients with MWS, episodic fevers are longer (several days) and patients can also develop chronic inflammatory complications such as aseptic meningitis, sensorineural hearing loss, and amyloidosis which may be fatal. Systemic inflammation in NOMID/CINCA is often persistent and associated with the most severe complications including bony overgrowth, visual loss and cognitive impairment. Signs and symptoms seen in NLRP3-AID spectrum are predominantly caused by the inappropriate release and action of IL-1β, since targeted therapies that block this cytokine are extremely effective in controlling the disease.

Autosomal-dominant inheritance is evident in about 75% of patients with MWS and FCAS whereas de novo *NLRP3* mutations account for CINCA/NOMID in more than half of affected children. The first clue that NLRP3-AID can also be caused by somatic *NLRP3* variants, and that only a small proportion of mutated cells are required for disease pathogenesis, came from a report in 2005 describing a 15 year old boy with CINCA/NOMID (31). The patient was found to have two different amino acid substitutions in *NLRP3*. One (p.S196N) was inherited from the unaffected mother, whilst the second (p.Y570C), was only detected in 16.7% of cells from whole blood and was thought to had arisen post-zygotically. The p.Y570C variant was determined to be pathogenic, since it had greater potential to induce ASC dependent NF-kB activation compared to the heterozygous p.S196N and the wild type gene. A subsequent case-control study demonstrated that somatic mosaicism is likely to be a major cause of CINCA/NOMID in patients who had previously tested negative for *NLRP3* mutations using conventional Sanger sequencing (32). The cellular/tissue distribution of mosaicism in these and subsequent similar cases was either not examined or was found to be ubiquitous, suggesting that mutations in *NLRP3* had arisen during early embryonic development (31-36).

The first report of a patient with late onset MWS-like disease with a known pathogenic mutation in *NLRP3,* but restricted to the myeloid-cell linage only, was published in 2015 (37). However, the authors were unable to determine if the mutation arose in the late stages of embryogenesis, after birth, or later in life during the development of myeloid progenitor cells. A subsequent report by a different group described another patient with late onset NLRP3-AID (symptom onset from age 56 years), in whom they were able to show that the somatic mutational event occurred at the stage of common myeloid progenitor cell differentiation (38). However, it was unclear from this report whether the mutation had arisen within the context of clonal expansion within bone marrow. A larger study which reported 8 patients with late onset NLRP3-AID (age range of disease onset 31-71 years) from a single centre, suggested that clonal expansion of myeloid cells carrying a pathogenic *NLRP3* mutation can occur over time. One of the patients from this cohort was found to have an increase in the frequency of the mutant allele from 5.1 to 45%, over a period of 12 years (39). Interestingly, this increased frequency coincided with the patient requiring more intense IL-1 inhibiting therapy. Although clonal expansion of myeloid cells was evident in this patient, it remains undetermined whether this phenomenon arises in the context of clonal non-malignant expansion of hematopoietic stem cells, such as clonal hematopoiesis of indeterminate potential (CHIP), or whether it is due to a selective survival advantage that NLRP3 mutations confer to myeloid cells. The former may provide favourable conditions for the acquisition of additional somatic mutations in alternate genes with proinflammatory effects (40). In terms of a potential survival advantage to myeloid cells, this is unlikely given the contributory role NLRP3 activation plays in the pathogenesis of a myelodysplastic syndrome causing inflammatory death of the progenitor cells (41, 42). The role of CHIP was explored in a single centre study of 6 patients, in which the presence of CHIP was tested using a custom-made, targeted panel. This study found that only 1 out of 6 patients carried pathogenic variants associated with CHIP. The affected patient

was found to have 3 mutations: one in DNMT3-A (c.2645G>A p.Arg882His) and two mutations in TET-2 (c.4585C>T p.Gln1529; p.Gln1699*), all with a significant VAF (0.407, 0.388 and 0.402 respectively)(43). These mutations were also associated with allelic skewing, which was determined by testing for X chromosome inactivation. The findings of this study suggest that CHIP is not a prerequisite for developing additional somatic mutations within the bone marrow.

The question of how a relatively small number of myeloid cells which carry pathogenic NLRP3 variants can cause a complete clinical phenotype of NLRP3-AID, remains unanswered. One potential explanation is that NLRP3 inflammasome complex activation is not confined to single cells, but rather that activated complex is released from cells via pyroptosis, remains enzymatically active and is taken up by surrounding cells, thus propagating the inflammatory cascade (44, 45). This argument is supported by the fact that soluble ASC protein specks can be detected in the serum of patients with NLRP3-AID and their concentration varies with disease activity (39, 46). This process might be coupled with more pathogenic *NLRP3* variants which are found predominantly in mosaic/somatic state but almost never as germline mutations, even though the acquired variants arise from mutation prone hotspots (47). This suggest that such variants are more harmful and likely to be embryonically lethal, if present in a germinal state.

Schnitzler's syndrome (SchS)

SchS is rare systemic adult-onset autoinflammatory disorder which, in common with VEXAS syndrome, has overlapping rheumatological and hematological features. However, in contrast to the recently described VEXAS syndrome, the genetic basis and aetiology of SchS remains unknown despite being identified as a clinical entity almost 50 years ago.

A diagnosis of SchS is dependent on a set of criteria of which the two major features are IgM paraprotein detection (or less commonly IgG) and the presence of chronic urticarial rash (48). The urticarial rash is identical to that observed in NLRP3-AID, being non itchy and with the unique histological features of neutrophilic urticarial dermatosis (30). Other similarities with NLRP3-AID include systemic symptoms such as fever and malaise and an extremely favourable symptomatic response to any form of anti-IL-1 inhibition. It is therefore not surprising that low grade somatic mutations in *NLRP3* were purported to play a role in the pathogenesis of SchS. However, across two studies with a total of 32 patients, no evidence of either somatic or germline pathogenic *NLRP3* variants was found (43, 46). Furthermore, 21 patients who were tested for 32 additional genes typically associated with SAID, were found not to carry any significant mutations (46).

A different explanation for the pathological and genetic basis of SchS comes from the observation that some patients with SchS later develop Waldenstrom's macroglobulinaemia (WM) (49). Around 90% of patients with WM have been reported to carry the somatic MYD88 p.L265P variant (50). Depending on the cellular location, this GoF variant can potentially have different effects. When found in B cells, the p.L265P variant is thought to drive the development of lymphoid malignancy via toll-like receptor signalling pathways (51). The same variant might have pro-inflammatory effects if located in the cells of myeloid linage, since MYD88 also has a role in propagating downstream signalling from the IL-1receptor (IL-1R). A recent report describing a new inflammatory disorder due to a germline GoF mutation in *MYD88,* characterized by a skin rash similar to that seen in SchS, supports this hypothesis (52). However, testing of 30 SchS patient for the p.L265P variant using ASO-PCR technique, which has a detection sensitivity down to 1% of the mutant allele, found that only 9/30 patients carried

the p.L265P mutation (43). This finding suggests that somatic MYD88 mutations might have a role in the pathogenesis of SchS but this is unlikely to be a universal mechanism. The same study also examined the role of CHIP in disease aetiology. CHIP can be associated with a pro-inflammatory bone marrow environment, certain chronic inflammatory diseases (53), and increased levels of inflammatory cytokines such as interleukins 1 (IL-1) and IL-6 (54). In addition, the CHIP-related somatic mutations *TET2* and *U2AF1*, encoding for transcription and splicing factors respectively, stimulate the generation of reactive oxygen species (ROS)(55). Consequently, ROS can trigger NLRP3 inflammasome formation as evidenced by the presence of ASC specks and pyroptosis (42, 56). Among 30 SchS patients, only 1 was found to have evidence of CHIP-associated mutation; this was a nonsense mutation in *STAG2* (c.559C>T p.Gln187Ter) with a low variant allele fraction (VAF) of 0.081 (43).

Another hypothesis for the pathogenesis of SchS relates to the role of paraprotein. There is no apparent relationship between the quantity of IgM paraprotein and severity of SchS or response to IL-1 inhibition (46). However, there are number of non-malignant or low-grade lymphoproliferative conditions where the paraprotein may have direct pathological role. For example, in cold agglutinin disease (CAD), IgM paraprotein has uniform epitope specificity for red blood cells and causes their destruction (57). A study examining the antigen specificity of IgM paraprotein in SchS patients found no evidence for a shared antigenic target, although the patients did have degree of skewed IGH repertoire, suggesting an underlying B cell clonality (58). A possible role for IgM paraprotein in the disease pathogenesis is suggested in two recent reports in which patients experienced disease remission following disappearance of the paraprotein, following either bone marrow transplant or treatment with a BTK inhibitor (59, 60).

Future work

Identifying the causes of SAIDs to date has predominantly focussed on germline, Mendelian inherited forms of disease, giving important insights into the mechanisms that underly autoinflammatory disorders (1). Although well studied in the context of cancers, research in to non-mendelian causes of SAIDs, in particular due to acquired (somatic) mutations, has proved more challenging. While we have reviewed the recent progress in this field, the authors acknowledge this progress is only a small step, although many lessons can be learned from them for future studies on AAIDs. For example, the recent finding of cell-type specific somatic VEXAS mutations argues against sequencing using solely DNA extracted from whole blood, but instead – akin to RNA studies – also using genomic DNA extracted from relevant cell types, where possible. This could increase the mutation allele frequency for low-level somatic mutations, compared to whole blood, and allow comparisons between disease vs non-disease relevant cells types to be made, providing greater variant detection and filtering power. While this approach has limitations, for example where mutations lead to death (or non-production) of disease-relevant cells, we recommend this as a next-step in AAID cases where existing genetic approaches have so far proved inconclusive. Furthermore, sequencing analysis pipelines which have predominantly focussed on detection of germline variants in cases of Mendelian disease, should be updated to include somatic-aware variant calling, such as MuTECT2. Finally, in cases where somatic mutations cannot be excluded, sequencing should be performed to an appropriate depth to allow calling of somatic mutations that may be present at a low frequency. Although the exact depth required depends on the expected allele frequency of the mutation, an average read depth of approximately 100x using PCR-free methods to prepare the sequencing libraries has been shown to improve the accuracy of somatic mutation detection compared to an average depth of 30x (61). While many exome- or panel-based sequencing pipelines may already meet this depth of coverage, most Genome sequencing is

currently performed to a lower average depth, e.g. 30x, which could miss low level somatic variants. Clinicians receiving genetic results should therefore be aware of these factors, particularly in cases where no mutation has been identified.

Summary

In summary, we have highlighted a number of exciting new developments in the field of acquired auto-inflammatory disorders and identified key learning points for future research. The surprising finding of *UBA1* mutations as the cause of a new acquired auto-inflammatory disease highlights the limited knowledge we have in this area and the scope there is for future research.

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Conflict of Interest

The authors declare no conflicts of interest.

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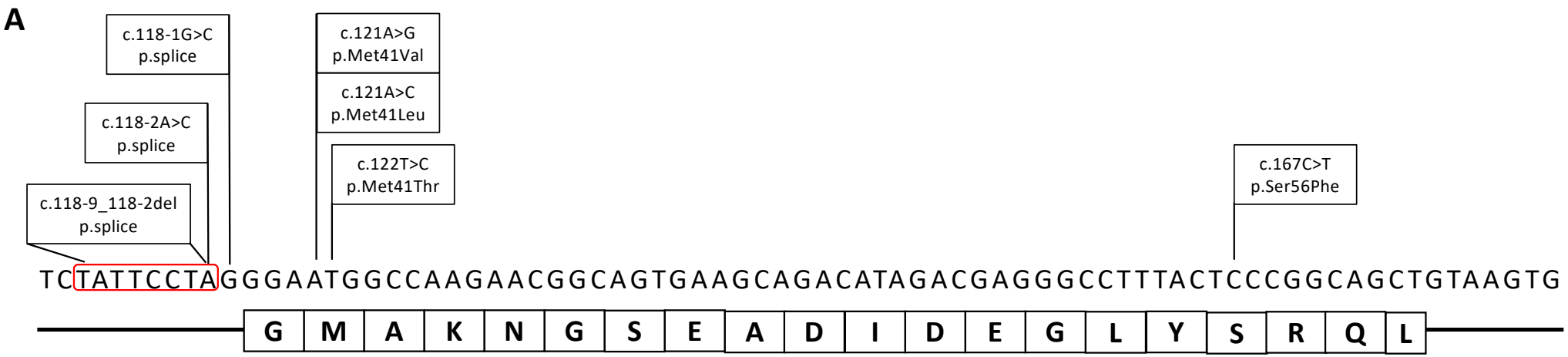
Legend to Figures

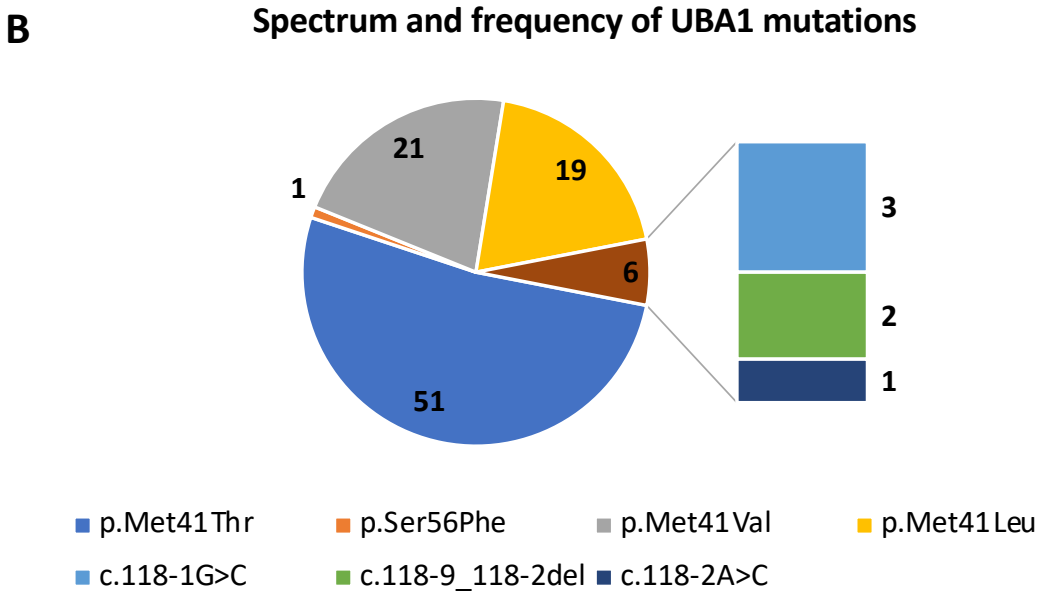
Figure 1 – Spectrum and frequency of *UBA1* mutations causing VEXAS.

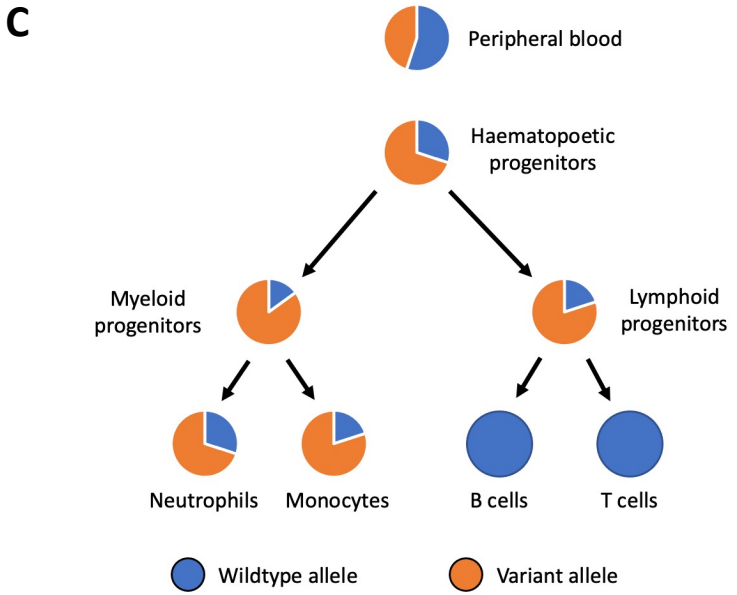
(A) Schematic of *UBA1* exon 3 showing the location of all known VEXAS mutations. All variants cluster at the p.Met41 and the splice acceptor site with the exception of p.Ser56Phe. Nomenclature based on *UBA1* transcript NM_153280.

(B) Frequency of known VEXAS mutations based on all available published mutations, summarised from Supplementary Table 1. The p.Met41Thr mutation accounts for over 50% of all known mutations.

(C) Allelic proportion of wild-type and VEXAS causing mutations in unsorted and sorted peripheral blood cells. Frequencies based on values in Beck et al., 2020.


A
c.118-1G>C
p.splice
c.118-2A>C
p.splice
c.118-9_118-2del
p.splice
c.121A>G
p.Met41Val
c.121A>C
p.Met41Leu
c.122T>C
p.Met41Thr
c.167C>T
p.Ser56Phe
TCTATTCCTAGGGAATGGCCAAGAACGGCAGTGAAGCAGACATAGACGAGGGCCTTTACTCCCGGCAGCTGTAAGTG
G M A K N G S E A D I D E G L Y S R Q L


B
Spectrum and frequency of UBA1 mutations
21
19
1
6
51
3
2
1
p.Met41Thr
p.Ser56Phe
p.Met41Val
p.Met41Leu
c.118-1G>C
c.118-9_118-2del
c.118-2A>C


C
Peripheral blood
Haematopoetic
progenitors
Myeloid
progenitors
Lymphoid
progenitors
Neutrophils
Monocytes
B cells
T cells
Wildtype allele
Variant allele

Supplementary Table 1 - Published Somatic UBA1 mutations.
All manuscripts identified based on a PubMed search of "VEXAS" (up to 08/07/2021)

Paper	Mutation (DNA)	Mutation (Protein)	Frequency	Diagnosis	Features	Comments
Beck et al., NEJM, 2020	c.122T>C	p.Met41Thr	15/25	VEXAS		
	c.121A>G	p.Met41Val	5/25	VEXAS		
	c.121A>C	p.Met41Leu	5/25	VEXAS		
Barba et al., Rheum., 2021	c.122T>C	p.Met41Thr	1/1	VEXAS	Patient is female	Monosomy X
Ferrada et al., Rheum., 2021	c.122T>C	p.Met41Thr	8	RP		
	c.121A>G	p.Met41Val	2	RP		
	c.121A>C	p.Met41Leu	3	RP		
Huang et al, Exp. Hem. and Onc., 2021	c.121A>C	p.Met41Leu	1/1	MDS		
Oganesyan, Rheum., 2021	c.118-1G>C	p.Splice	1/1	VEXAS		
Poulter et al., Blood, 2021	c.122T>C	p.Met41Thr	5	VEXAS		
	c.121A>G	P.Met41Val	3	VEXAS		
	c.167C>T	p.Ser56Phe	1	VEXAS		
	c.118-1G>C	p.Splice	1	VEXAS		
Rieu et al., Brit. J. Haem. 2021	c.121A>C	p.Met41Leu	1/1	VEXAS		
Sakuma et al., Rheum., 2021	c.122T>C	P.Met41Thr	1/1	VEXAS		
Tsuchida et al., Ann. Rheum. Dis., 2021	c.122T>C	p.Met41Thr	3/16	RP		1 female had low level somatic variant
	c.121A>C	p.Met41Leu	3/16	RP		
	c.121A>G	p.Met41Val	2/16	RP		
Van der Made et al., JACI, 2021	c.121A>G	p.Met41Val	4/12	VEXAS		
	c.122T>C	p.Met41Thr	7/12	VEXAS		

	c.121A>C	c.Met41Leu	1/12	VEXAS		Retrospective study
Takahashi et al., Arth. & Rheum., 2021	c.121A>G	p.Met41Val	1/1	VEXAS		
Himmelmann et al., EJCRIM, 2021	c.121A>C	p.Met41Leu	1/1	VEXAS		
Temple et al., Rheum., 2021	c.118-9_118-2del	p.splice	2/2	VEXAS		
Magnol et al., Rheum., 2021	c.122T>C	p.Met41Thr	1/1	Spondyloarthritis		
Grey et al., JCI, 2021	c.122T>C	p.Met41Thr	1/1	VEXAS	Also hemophagocytic lymphohistiocytosis	
Bourbon et al., Blood, 2021	c.121A>G	p.Met41Val	3/19	VEXAS		
	c.121A>C	p.Met41Leu	1/19	VEXAS		
	c.122T>C	p.Met41Thr	5/19	VEXAS		
	c.118-2A>C	p.Splice	1/19	VEXAS		
	c.118-1G>C	p.Splice	1/19	VEXAS		
Lee et al., Rheum, 2021	c.121A>C	p.Met41Leu	1/1	VEXAS		
Staels et al., Front. Immun., 2021	c.122T>C	p.Met41Thr	1/2	VEXAS		
	c.121A>C	p.Met41Leu	1/2	VEXAS		
Ross et al., J Rheum, 2021	c.122T>C	p.Met41Thr	1/1	VEXAS	ANCA-associated vasculitis	
Gurnari et al., Blood, 2021	c.122T>C	p.Met41Thr	2/9	VEXAS		
Arlet et al., NEJM, 2021	c.121A>G	p.Met41Val	1/2	VEXAS	Female case	Monosomy X
	c.121A>C	p.Met41Leu	1/2	VEXAS	Female case	Monosomy X

*RP = relapsing polychondritis