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Genetic Diagnosis Of Vexas Syndrome: A New Rare And Deadly Autoinflammatory Disorder In Adults

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Abstract

VEXAS (Vacuoles, E1 enzyme, X linked, autoinflammatory, somatic syndrome) syndrome is a rare autoimmune condition that can be fatal in adult persons. VEXAS syndrome is classified as an autoinflammatory disease. This syndrome typically affects older adults, primarily males, with signs and symptoms of the condition developing in a person's fifties, sixties, or seventies. About 1 in every 13,591 adults may have the condition. Mutations affecting methionine-41 (*p. Met41*) in *UBA1* of blood cells, the major *E1* enzyme that initiates ubiquitylation is responsible for VEXAS. The peripheral-blood exome sequence data was analysed, independent of clinical phenotype and inheritance pattern to identify deleterious mutations in ubiquitin-related genes. Sanger sequencing, immunoblotting, immunohistochemical testing, flow cytometry, and transcriptome and cytokine profiling were performed. CRISPR-Cas9-edited zebrafish were used as an *in vivo* model to assess the gene function. In such patient, treatment-refractory inflammatory syndrome develops in late adulthood, with fevers, cytopenias, characteristics vacuoles in myeloid and erythroid precursor cells, dysplastic bone marrow, neutrophilic cutaneous and pulmonary inflammation, chondritis, and vasculitis. Patients can also have a shortage of blood cells called platelets. Treatments include corticosteroids, immunosuppressants and sometimes a bone marrow transplant. In the future, VEXAS syndrome along with other related autoinflammatory conditions, and maybe other hemato-inflammatory diseases, in adulthood may change our perception of the already supposedly known rheumatic or systemic autoimmune diseases, and genetic diagnosis may become a routine clinical practice for physicians in experienced referral centres.

Keywords: Autoinflammatory disease, Genetic diagnosis, Systemic autoimmune diseases, Ubiquitylation, UBA1, VEXAS syndrome.

1. Introduction:

Vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic (VEXAS) syndrome is an autoinflammatory disorder that has just recently been discovered to have a corresponding missense somatic X chromosomal mutation (Mohammed et al., 2023). The *UBA1* gene, which codes for the principal enzyme of cellular ubiquitylation, was shown to have acquired mutations in all cases of VEXAS using a genotype-first strategy to illness identification (Grayson et al., 2021). The methionine 41 of the E1-ubiquitin ligase *UBA1* is affected by a somatically acquired mutation that causes the development of a catalytically deficient isoform that causes inflammation, resulting in this disease (Bourbon et al.,2021). Through the entire exome sequencing of 2,560 patients, the VEXAS syndrome was discovered in 2020. During this process, three patients had a mutation in the ubiquitin activating enzyme 1 *(UBA1)* gene. Histopathology and illness began to exhibit a common pattern. The majority of these cases were older males with cytopenias and multi-system inflammatory symptoms (Al-Hakim et al., 2023). The term VEXAS is based on the main characteristics of the syndrome. In bone marrow aspirates, vacuoles can be detected in myeloid and erythroid progenitor cells. E1 enzyme or the ubiquitin activating enzyme, or is represented by the X-linked gene *UBA1* (Grayson et al., 2021). It was discovered that the same mutation was present in systemic autoinflammatory diseases (SAID), discrete inflammatory diseases, and myelodysplastic syndrome (MDS) (Al-Hakim et al., 2023). However, VEXAS syndrome is a new type of autoinflammatory illness in which the causal mutation is a somatic mutation that is acquired later in life. No matter the variant allele percentage of the altered cells, the disease penetrance linked to the known pathogenic mutations in UBA1 seems to be near to 100%. A gene study using next-generation sequencing (NGS) is frequently the only way to detect this somatic mosaicism (Poulter and Savic, 2021). The success of these novel modalities and strategies is highlighted by VEXAS, opening the door for potential future breakthroughs (Al-Hakim et al., 2023).

1.1 Genetics of VEXAS:

On X chromosome the *UBA1* gene, which causes VEXAS syndrome, is affected by somatic postzygotic mutations (Beck et al., 2020). It is well known that somatic mutations occur over time, and their effects might include the development of benign allele variations, carcinogenesis, and clinical illness (Mustjoki et al., 2021). Over 90% of the activation of ubiquitin, ubiquitylation-dependent intracellular protein degradation, and cell homeostasis are carried out by the primary E1 activating enzyme encoded by the gene *UBA1* in humans (Callis, 2014; Moudry et al., 2012). In patients with germline mutations, the *UBA1* (Ubiquitin-Like Modifier-Activating Enzyme 1), encoding one of the two E1 enzyme isoforms that initiates ubiquitylation in the cell's cytoplasm, has been related to X-linked infantile spinal muscular atrophy. On the other hand, acquired *UBA1* gene mutations in blood cell progenitors are linked to a particular and distinct systemic inflammatory entity (Beck eta al., 2020). The methionine at amino acid position 41 is the site of the *UBA1* gene's most often seen alterations. Currently, p.Met41Thr (c.122T > C), p.Met41Val (c.121A > G), and p.Met41Leu(c.121A > C) account for the most frequently occurring pathogenic variants; however, p.Ser56Phe(c.167 C > T), and p.Gly40_Lys43del(c.118-1G > C), which are respectively likely pathogenic and pathogenic, have also been identified (Georgin-Lavialle et al., 2022; Shaukat et al., 2022). Due to its X-linked expression, this condition is typically only seen in males; however, in some cases, such as one involving a 67-year-old patient with Turner syndrome, X0 monosomy may be to blame (Stubbins et al., 2022). *UBA1b*, a shorter cytoplasmic isoform initiated at p.Met41, or *UBA1a*, a nuclear isoform initiated at *p.Met1*, are the two possible forms of expression for the gene (Patel et al., 2021).

1.2 Clinical description:

The clinical characteristics of VEXAS, a severe, developing disease, overlap those of rheumatologic and hematologic disorders (Grayson et al., 2021). Since inflammation can affect any organ or tissue, the clinical manifestations of VEXAS syndrome are incredibly diverse (Matsumoto et al., 2022). In fact, VEXAS symptoms might resemble a variety of systemic rheumatic diseases linked to myelodysplastic syndrome MDS (hematologic malignancy), such as small vessel vasculitides, rheumatoid arthritis, seronegative spondyloarthritis, Sweet syndrome, relapsing polychondritis (RP), polyarteritis nodosa, and even Behcet's disease (Mekinian et al., 2016; de Hollanda et al., 2011). VEXAS patients experience a variety of hematologic issues, such as macrocytic anemia, thrombocytopenia, thromboembolic illness, and progressive bone marrow failure that can lead to hematologic malignancy (Grayson et al., 2021).

1.3 Symptoms & inflammation

Due to the disease's wide variety of symptoms, which commonly resemble those of other illnesses, VEXAS syndrome was challenging to diagnose. They frequently include fever, weight loss, exhaustion, nocturnal sweats, and muscle aches. The illness can also manifest itself in a variety of ways on the skin, eyes, bone marrow, lungs, joints, and blood vessels, Cardiovascular system, Gastrointestinal tract Kidneys and urogenital system within a person. Additionally, those who have VEXAS syndrome may see a gradual decline in their blood levels, which can be deadly if untreated. Up to 40.5% of cases involve the eyes, most commonly due to episcleritis, uveitis, scleritis, orbital mass, and blepharitis. There may be periorbital and orbital irritation. About 11% of individuals will experience heart involvement, which may manifest as myocarditis, pericarditis, or even develop into cardiomyopathy (Vitale et al., 2023).

1.4 Genetic Diagnosis & treatment

Genetic testing is used to make a VEXAS diagnosis, and this testing looks for abnormalities in the X chromosome's *UBA1* gene. VEXAS syndrome is diagnosed using a multidisciplinary approach that includes, among others, rheumatologists, immunologists, haematologists, geneticists, pathologists, ophthalmologists, dermatologists, internal medicine specialists, geriatricians, and radiologists. The diagnosis is based on the correct interpretation and combination of clinical manifestations and laboratory features. The detection of vacuoles in bone aspirate smears and the detection of MDS or other haematological disorders during laboratory evaluation or bone marrow biopsy constitute crucial steps in the diagnosis (Vitale et al., 2023). By examining peripheral-blood exome sequence data irrespective of clinical manifestation and inheritance pattern to find harmful mutations in ubiquitin-related genes. Sanger sequencing, immunoblotting, immunohistochemical analysis, flow cytometry, and transcriptome and cytokine profiling were all carried out. Zebrafish that had undergone *CRISPR-Cas9* editing were employed as an *in vivo* model to analyse gene function (Beck et al., 2020).

1. 4. 1 Bone marrow aspiration and biopsy

A bone marrow biopsy can allow a hematologist to examine a patient's blood more closely. The visible vacuoles or bubbles in blood that make up the "V" in VEXAS syndrome are a typical symptom of the condition. During the diagnostic process for VEXAS syndrome, the specific changes discovered by the histopathologic analysis of the bone marrow are crucial. The typical hematologic findings in VEXAS patients include macrocytic anaemia, thrombocytopenia, marked hypercellular bone marrow with granulocytic hyperplasia, megaloblastic changes in erythroid precursors, the absence of hematogones, as well as prominent vacuoles (Li et al., 2022). Hyperplasia, megakaryocytic atypias, and hemophagocytosis are some of the signs that can be seen during a bone marrow biopsy that point to MDS. With an average of 5-7 vacuoles per cell, 15% of myeloid and erythroid cells have vacuoles. Eosinophils, monocytes, plasma cells, and megakaryocytes all have vacuoles, as well.

1.4.2 Radiologic examination

A chest X-ray, followed by computed tomography (CT), is required to identify lung inflammatory involvement and deep lymph node enlargement in VEXAS patients. Strong ultrasound Doppler may aid in the detection of thrombotic vascular events (Li et al., 2022). Positron emission tomography (PET) scan can detect a diffuse increase in fludeoxyglucose 18F uptake in the bone marrow, spleen, and thyroid gland and also show nodules in the lungs, pancreas, parotids, and other organs, which may aid in the diagnosis of onco-hematological VEXAS-related illnesses (van der Made et al., 2022).

1.4.3 Treatment

Understanding the molecular basis of a disease is a critical first step in finding more effective treatments. The VEXAS syndrome is associated with significant morbidity and mortality. Symptoms are often resistant to treatment. Many VEXAS patients will take anti-inflammatory medications called glucocorticoids to help manage symptoms, but those can often have unwanted steroid side effects. *Azacytidine*, a hypomethylating drug, was taken for the longest median time (21.9 months), although there was no change in cytopenia or myelodysplastic characteristics on bone marrow. Janus kinase inhibitors were beneficial for some aspects of systemic inflammatory illness, particularly skin involvement (Grayson et al., 2021).

Future Direction

The greater awareness of the VEXAS syndrome will improve illness understanding and may broaden the present phenotypic. A targeted and automated examination of cohorts with a high pretest probability could be

a helpful strategy in the future. In the future, VEXAS syndrome and other closely related autoinflammatory conditions, possibly other hemato-inflammatory diseases, in adulthood, may alter our perception of the supposedly well-known rheumatic or systemic autoimmune diseases, and genetic diagnosis may become a standard clinical practice for doctors in knowledgeable referral centers. Future identification of systemic inflammatory diseases caused by somatic mutations could lead to the distinction of current entities, as multiple rheumatological conditions are diagnosed based on clinical features, potentially reopening old cases (Ruffer and Krusche, 2023).

Conclusion

The VEXAS syndrome is a refractory inflammatory condition that affects elderly men and results in systemic inflammation and hematologic abnormalities. Except for high-dose glucocorticoids, no effective treatment has been discovered. It may be successful to target and eliminate UBA1 mutations, stop the inflammatory cascade reaction, or restore UBA1b function. Gene editing treatments and bone marrow transplants may offer a cure. Through allogeneic bone marrow transplant, the condition can be diagnosed early. Adult patients with systemic inflammation, hematologic diseases, and vacuolation of myeloid and erythroid precursor cells should be tested for UBA1 mutations to help with early diagnosis and a better prognosis.The syndrome will require extensive research to evaluate genetic variants, their association with other genes, and understand clinical behaviour and therapeutic approaches.

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