A novel *AGBL3* variant potentially associated with hypocomplementemic urticarial vasculitis syndrome: examining clinical outcomes and therapeutic responses

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Abstract

Objective

Hypocomplementemic urticarial vasculitis syndrome (HUVS) is a rare and severe form of urticarial vasculitis (UV), and characterised by chronic urticaria, systemic vasculitis, and hypocomplementemia. Pathogenesis of HUVS is unknown. Genetics may play a role, and DNASE1L3 gene variants were identified in 2 families with HUVS.

Methods

In this study, we conducted a trio-based whole exome sequencing (WES) study to identify new candidate gene(s) in a consanguineous family with an affected son with HUVS, and the identified variant was confirmed by Sanger sequencing. Functional significance of the variant was assessed by bioinformatic tools, including molecular dynamics (MD).

Results

The patient had recurrent episodes of fever, urticarial rash, red eyes, and joint pain from age 13 along with elevated acute phase response, and increased ANA titres over time. WES analysis revealed a nonsense c.769C>T (p.Gln257Ter) variant in the AGBL3 gene, heterozygous in the parents, and homozygous in the index case. The comprehensive MD analyses demonstrated that the Gln257Ter truncation not only eliminates a portion of the protein but also fundamentally alters the structural and dynamic properties of the remaining regions.

Conclusion

Our results indicate that AGBL3 is a novel candidate gene potentially associated with the pathogenesis of HUVS.

Key words

vasculitis, hypocomplementemic urticarial vasculitis syndrome, AGBL3, variation

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Received on January 14, 2025; accepted in revised form on August 1, 2025.

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Funding. This study was funded by the Scientific Research Projects Coordination Unit of Istanbul University (Project number TYL-2020-36201).

Competing interests: C. Bracaglia is a consultant for SOBI and Novartis, and is member of speaker's bureau for GSK. All other authors declare no competing interests.

Introduction

Hypocomplementemic urticarial vasculitis syndrome (HUVS) is a rare autoimmune disorder with an incidence of 0.5/100.000 and characterised by urticaria for at least six months, low complement levels, and systemic findings such as arthritis, pulmonary disease, and ocular inflammation (1, 2-5). Existing research indicates that HUVS is present in 7-8% of systemic lupus erythematosus (SLE) patients, and over half (54%) of HUVS patients received the diagnosis of SLE during follow-up (3-6).

Elevated levels of anti-C1q antibodies are detected in most of the patients, which may bind to the collagen domain of C1q, forming immune complexes that deposit on vascular walls, leading to complement activation and endothelial damage (2). Some HUVS patients had variations in the *DNASE1L3* gene, which is also associated with monogenic SLE, indicating the hereditary forms of HUVS (6, 7).

This study aimed to investigate new candidate genes associated with HUVS using trio-based whole exome sequencing (WES) in a consanguineous family with one affected child.

Materials and methods

Participants

The study group comprised the consanguineous family of an index case and his parents, as well as two unrelated paediatric patients with HUVS from Italy. The index case and parents were followed in the Division of Rheumatology at Istanbul Faculty of Medicine. Local Ethics Committee approval was obtained (122249, 21/06/2019), and all provided written informed consent.

DNA isolation and sequencing

Peripheral blood samples were obtained in 5 ml EDTA-containing tubes, and total DNA was isolated. WES was performed at Oxford Gene Technology (Oxfordshire, UK) on the Illumina HiSeq2000 platform using TruSeq v3 chemistry. The identified *AGBL3* variant was verified using Sanger sequencing.

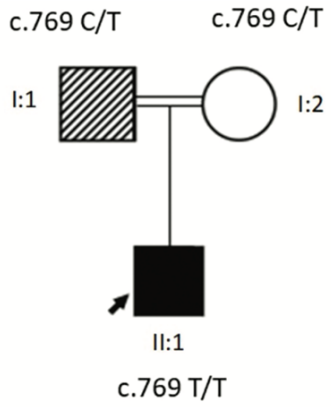
Bioinformatics for determining the effect of the variation on protein structure

The 3D structure of native and truncated AGBL3 protein was investigated first using standard bioinformatics tools (8-12). The native protein was modelled via homology modelling in SWISS-MODEL and validated with PROCHECK. The truncated form was generated using SWISS PDB Viewer. NOMAD-Ref tool, which uses the Gromacs algorithm by default, was used to optimise protein models for conformational energy minimisation. SPPIDER and Polyview3D were used to predict solvent accessibility and secondary structures. SRide was used to identify stabilising residues, and FlexPred to predict residue positions in conformational switches. HBAT was used for analysis of unbound interactions.

The structures of full-length AGBL3 and its Gln257Ter variant were generated using AlphaFold and prepared for molecular Dynamics (MD) simulations. Details of the MD analyses are given in the Supplementary Figures 1-5. Both systems underwent three independent replicates of 100 ns production runs, which were used in the analysis. The simulations were performed using NAMD3.b54. All frames in each trajectory were aligned to the first frame of the simulation by using VMD 1.9.4 before performing the analysis. The MD analyses included Root Mean Square Deviation (RMSD) and Fluctuation (RMSF), Contact Frequency, and Comparative Residue Flexibility.

Results

The index case was a 22-year-old male (Fig. 1). The patient experienced recurrent episodes of fever, urticarial rash on the extremities and trunk, conjunctival injections, and arthralgia starting at age 13. The 2-3 day-lasting attacks became more frequent during warm weather conditions or after hot baths. Histopathological analysis of the cutaneous lesions confirmed the diagnosis of urticarial vasculitis. During the episodes, he exhibited markedly elevated levels of C-reactive protein and erythrocyte sedimentation rate, and his acute phase response did not normalize between



Number	Diagnosis	c.769 C>T
I:1	Mucocutaneous Behçet's	Heterozygous (C/T)
I:2	Healthy	Heterozygous (C/T)
II:1	HUVS	Homozygous (T/T)

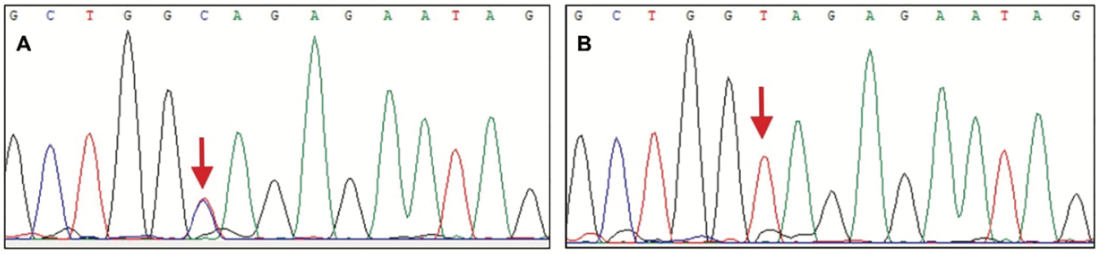


Fig. 1. Family tree, zygosity, clinical features, and DNA sequencing results.
I:1: Father with mucocutaneous Behçet's; I:2: Healthy mother; II:1: Index case with HUVS. The *AGBL3* gene c.769 C>T variant results in p.Gln257Ter, a truncated protein. Sanger sequencing results of AGBL3 gene for c.769C>T variant are given in the bottom part of the Figure (**A**: heterozygous (C/T) patient I:1; **B**: homozygous (T/T) patient II:1. c.769C>T variation marked with red arrow).

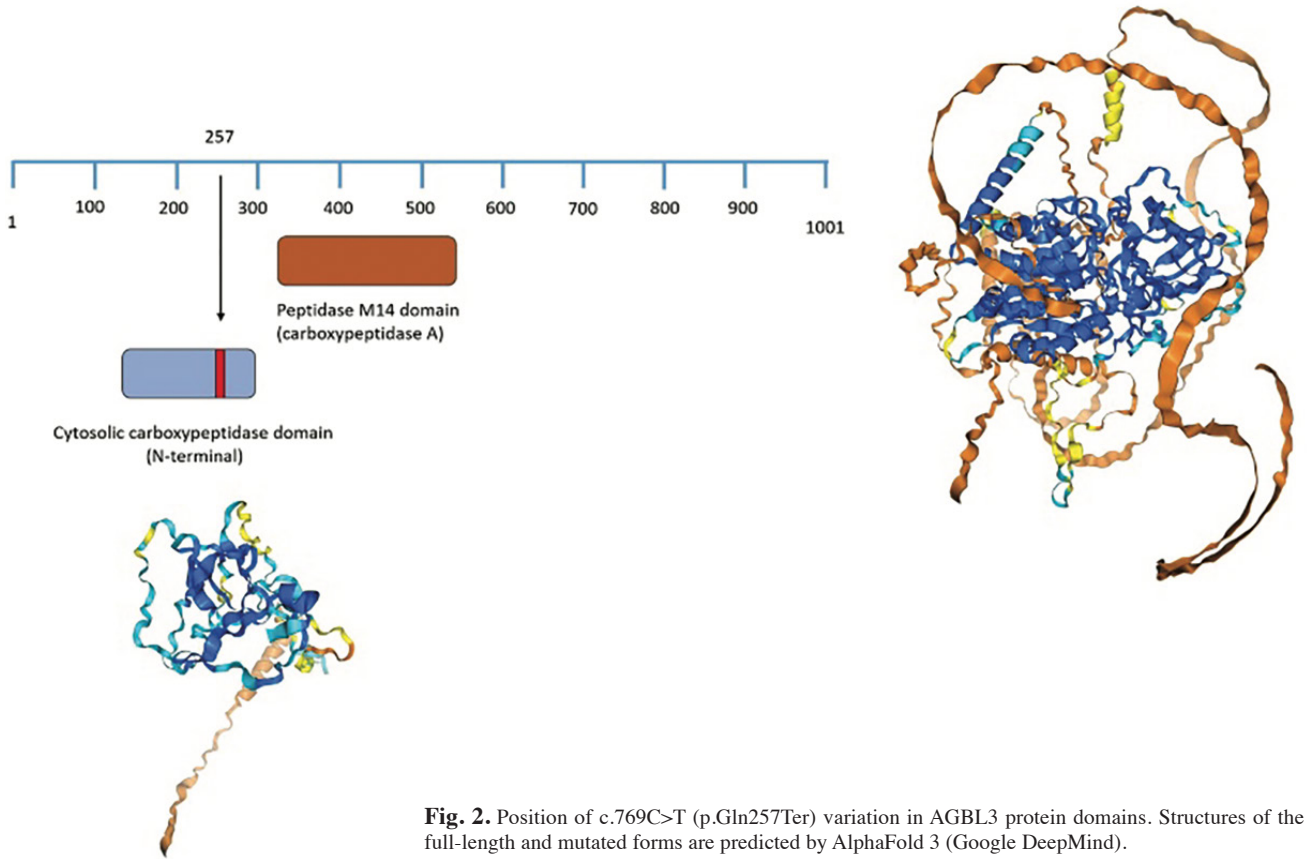


Fig. 2. Position of c.769C>T (p.Gln257Ter) variation in AGBL3 protein domains. Structures of the full-length and mutated forms are predicted by AlphaFold 3 (Google DeepMind).

the flares. Low complement C3 and C4 levels were observed even during asymptomatic periods. The patient's ANA test became positive during the disease course, with increasing titres over time. The patient exhibited a partial response to glucocorticoid therapy, and no clinical or laboratory improvements were seen with anti-IL-1 treat-

ments (canakinumab and anakinra). He experienced a macrophage activation syndrome following a viral pneumonia and required intensive care support. After the observation of increased titres of ANA, rituximab treatment was administered, and a favourable outcome was observed. The patient experienced fewer and less severe attacks, which were only triggered by infections, and the acute phase reactants almost normalized during the quiescent periods. During the pandemic, his rituximab treatment was stopped, and he was followed with azathioprine and glucocorticoids.

Both parents were second-degree cousins. His 55-year-old father exhibited mucocutaneous manifestations of Behçet's disease and was treated with colchicine, while his 48-year-old mother was asymptomatic.

Exon sequencing of the family revealed a homozygous nonsense c.769C>T (p.Gln257Ter) variation in the *AGBL3* gene of the index case, and both parents were identified as heterozygous carriers. The variant was further confirmed with Sanger sequencing (Fig. 1). Allele frequency of the variant was reported as extremely low 6.445 x 10^{-7} in the GnomAD database (chr7-134719111 C>T). This nonsense variation results in premature termination of the protein and deletion of the functional carboxypeptidase domain of the AGBL3 protein (Fig. 2). The patient and parents were negative for the *DNASE1L3* variants.

This variation was also screened by Sanger sequencing in two unrelated paediatric cases (patients 4 and 5) diagnosed with HUVS from Italy, who were negative for the *DNASE1L3* variants, and both were negative for the p.Gln257Ter variant. Sequencing analysis of the other functional regions (exons 2-10) of the *AGBL3* gene revealed no variation, other than common polymorphisms.

Bioinformatic analyses showed that this premature termination is associated with significant alterations in the structure and stability of AGBL3. The native and early terminated AGBL3 protein was modelled the SWISS-MODEL and AlphaFold2 (Fig. 2). SPPIDER's analysis revealed differences in the secondary structure between truncated AGBL3 models and their native counterparts (Supplementary Table S1). The significant change in secondary structure predicted a possible change in protein folding and intracellular interactions. All stabilising residues present in the native AGBL3 protein model were deleted in truncated form (Supplementary Table S2). The flexible residues of the mutant and wild-type proteins exhibited differences (Supplementary Table S3). A remarkable decrease in the strong H-bond N-H..O and a decrease in weak H-bonds such as C-H.O and C-H.N were predicted in the truncated protein by HBAT mediation (Supplementary Table S4).

MD analyses revealed distinct residue-residue interactions and contact patterns between wild-type and p.Gln257Ter, demonstrating that the truncation fundamentally alters the folding of the protein (Supplementary File). The new contact regions and alternative folding patterns led to the altered conformational behaviour in the Principal Component Analysis plots. This comparison reveals regions with differential flexibility between the two systems, with some segments showing increased mobility in the Gln257Ter variant suggesting that these regions become more dynamic in the absence of stabilizing interactions from the rest of the protein, while others display reduced fluctuations, possibly indicating formation of new, non-native interactions that restrict movement. The overall trend of increased flexibility in the truncated variant is consistent with a less structured and more dynamic conformation.

Discussion

The pathogenic mechanisms associated with HUVS have not been fully understood, but genetic factors are considered to play an important role. *DNASE1L3* variants were identified in two familial cases of HUVS (6, 7). In the current study, analysis of a consanguineous family with one affected patient with HUVS revealed no pathogenic variant in the *DNASE1L3* gene, but we identified a novel potentially pathogenic variant in the *AGBL3* gene (c.769C>T, p.Gln257Ter). The premature termination of the AGBL3 protein leads to the deletion of the cytosolic carboxypeptidase (CCP) domain, which may play a role in protein folding, regulation, and/or binding to other proteins (13). The c.769C>T variant can also lead to nonsense-mediated decay, where the prematurely terminated polypeptide may be degraded through the proteasomal degradation pathway. Alternatively, the truncated protein may persist in the cell in truncated form. Bioinformatic analyses revealed that the truncated protein exhibits a significant reduction in coil and beta secondary structures, as well as a deletion of the helix structure. The comprehensive MD analyses demonstrated that the Gln257Ter truncation not only eliminates a portion of the protein but also fundamentally alters the structural and dynamic properties of the remaining regions. These changes likely have significant implications for protein function, particularly regarding substrate recognition, binding partner interactions, and catalytic activity. These results need to be confirmed by further functional studies and *in vitro* experiments.

AGBL3 is an enzyme catalysing deglutamylation and deaspartyllation, especially on the C-terminal tail of tubulin, affecting the terminal tyrosine, which stabilizes microtubules. By removing the penultimate glutamate, AGBL3 can create irreversible Δ2-tubulin, a characteristic feature of stable microtubules in neurons, centrosomes, and cilia. Tubulins have been linked to autoinflammatory and autoimmune diseases, and microtubules play a role in forming the NLRP3 inflammasome (14). The N-terminal portion of pyrin also binds to microtubules. Pyrin is thought to regulate inflammation by interacting with the cytoskeleton in granulocytes and monocytes. Colchicine, an alkaloid known to function by binding to tubulin and preventing the elongation of microtubules, is frequently used in conditions associated with pyrin inflammasome (14).

In conclusion, the novel early termination variant in the *AGBL3* gene iden-

tified in this study appears as a candidate gene potentially associated with the pathogenesis of HUVS. Additional research is needed to better understand the clinical features associated with *AGBL3* gene variants, which may also help to clarify the heterogeneous pathogenesis of HUVS.

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