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HETEROZYGOUS PATHOGENIC *MASP2* VARIANT ASSOCIATED WITH INFANTILE GIANT CELL HEPATITIS WITH AUTOIMMUNE HAEMOLYTIC ANAEMIA IN A CHILD

OTOİMMÜN HEMOLİTİK ANEMİLİ İNFANTİL DEV HÜCRELİ HEPATİTLİ BİR ÇOCUKTA HASTALIKLA İLİŞKİLİ HETEROZİGOT PATOJENİK *MASP2* VARYANTI

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ABSTRACT

Objective: Infantile giant cell hepatitis with autoimmune haemolytic anaemia (GCH-AHA) is a rare disease characterised by giant cell and autoimmune haemolysis. The pathogenic mechanisms involve several factors, including genetic and immunological components, particularly those related to the lectin pathway of the complement system. In this study, we aimed to identify possible germline variations in patients with GCH-AHA.

Material and Method: Whole-exome sequencing (WES) was performed on a 6-month-old boy who was diagnosed with GCH-AHA. An in-house data analysis pipeline was applied to determine familial segregation using Sanger sequencing. ELISA was used for MASP2 protein detection.

Result: WES revealed a likely pathogenic heterozygous missense variant (p.(Cys618Tyr)) in the mannose-binding lectin (MBL)-associated serine protease-2 (*MASP-2*) gene. The *MASP2* variant identified in the serine protease domain was predicted to disrupt disulphide bonds. *In vitro* assays showed decreased MASP2 levels in the patient and mother compared with controls, supporting the potential pathogenicity of the variant.

ÖZET

Amaç: Otoimmün hemolitik anemili infantil dev hücreli hepatit (GCH-AHA), dev hücre ve otoimmün hemoliz ile karakterize nadir bir hastalıktır. Patojenik mekanizmalar, genetik ve immünolojik bileşenler, özellikle de kompleman sisteminin lektin yolağı ile ilgili olanlar dahil olmak üzere çeşitli faktörleri içerir. Bu çalışmada GCH-AHA'daki olası germ hattı varyasyonlarını analiz etmeyi amaçladık.

Gereç ve Yöntem: GCH-AHA tanısı konan 6 aylık bir çocukta tüm ekzom dizileme (TED) yapıldı. In house veri analizi algoritması uygulandı ve Sanger sekanslama ile ailesel segregasyon belirlendi. MASP2 protein tespiti için ELISA kullanıldı.

Bulgular: TED, mannoz bağlayıcı lektin (MBL) ile ilişkili serin proteaz-2 (*MASP-2*) geninde muhtemel bir patojenik heterozigot yanlış anlamlı varyantı (p.(Cys618Tyr)) ortaya çıkardı. Tahmin araçları bulgularına göre, serin proteaz domainde bulunan *MASP2* varyantının disülfit bağlarını bozduğu tahmin edilmiştir. In vitro testler, hastada ve etkilenen annede MASP2 seviyelerinin kontrollere kıyasla azaldığını göstererek varyantın potansiyel patojenitesini desteklemiştir.

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Conclusion: This study highlighted the association between a novel *MASP2* variant and GCH-AHA, emphasising the role of the lectin pathway in the pathogenesis of this rare disorder. The variable expressivity and incomplete penetrance observed in MASP2 deficiency underscore the complexity of genotype-phenotype correlations. Further investigations into the lectin pathway's detailed activation and its impact on GCH-AHA pathogenesis are warranted for a comprehensive understanding of the disease mechanisms.

Keywords: Infantile giant cell hepatitis, autoimmune haemolytic anaemia, complement system, MASP2, whole-exome sequencing

Sonuç: Bu çalışma, yeni bir *MASP2* varyantı ile GCH-AHA arasındaki ilişkiyi vurgulayarak, bu nadir bozukluğun patogenezinde lektin yolunun rolünü vurgulamaktadır. MASP2 eksikliğinde gözlemlenen değişken ifade ve eksik penetrasyon, genotip-fenotip korelasyonlarının karmaşıklığının altını çizmektedir. Lektin yolunun ayrıntılı aktivasyonu ve bunun GCH-AHA patogenezi üzerindeki etkisine ilişkin daha fazla araştırma, hastalık mekanizmalarının kapsamlı bir şekilde anlaşılması için önem arz etmektedir.

Anahtar Kelimeler: İnfantil dev hücreli hepatit, otoimmün hemolitik anemi, kompleman sistemi, MASP2, tüm ekzom dizilimi

INTRODUCTION

Infantile giant cell hepatitis with autoimmune haemolytic anaemia (GCH-AHA) is a progressive disorder characterised by diffuse giant cell hepatocyte transformation and autoimmune haemolysis (1, 2). Patients diagnosed within the first two years of life with elevated aminotransferase levels, acute liver injury, and haemolytic anaemia (3). Infection susceptibility is common, with 25% of patients harbouring infections such as mycoplasma, Epstein–Barr Virus (EBV), and acute otitis media.

The aetiology of GCH-AHA is poorly understood; approximately 49% of patients have idiopathic disease. The clinical presentation of GCH-AHA varies. Biliary atresia, immune dysregulation, neonatal hemochromatosis, and hypopituitarism have been seen in the patients (4). Although the genetic background is unknown, there is strong evidence of the role of humoral immunity and the complement system. Patients are Coombs-positive for immunoglobulin G positive (IgG+), implying involvement of complement protein 3 (C3) and immunoglobulins. In addition, complement-mediated damage and C5-9-mediated hepatocyte injury have been observed in children with GCH-AHA (5, 6).

The complement system is a system within the natural immune system that is activated in three ways: classical, alternative, and lectin pathways (7). When the lectin pathway (LP) is activated, the pathogen is recognised by its mannose moieties via mannose-binding lectin (MBL) and/or ficolin, and this engagement activates the MBL-associated serine proteases: MASP1, MASP2, and MASP3. MASPs cleave the complement system; autoactivation of MASP2 triggers cleavage of complement factors C4 and C2 and generates C3 convertase (8). MASP2 is the key mediator of LP because MASP2 is the only serine protease capable of cleaving C4 (9). MASP2 can initiate activation of the lectin pathway without the contribution of other proteases.

The Inborn Errors of Immunity Committee (International Union of Immunological Societies (IUIS) Primary Immune Deficiency Expert Committee) considered autosomal recessive MASP2 deficiency as an inherited complement deficiency (10). MASP2 deficiency (MIM:605102) is a rare disorder with a prevalence of <1:1,000,000. This disorder was first described in 2003 in an adult patient with severe recurrent pneumococcal infections in addition to autoinflammatory and autoimmune diseases. This patient carried the biallelic *MASP2*:p.(D120G) variant, which significantly reduced serum MASP2 levels (11). Current studies show that MASP2 deficiency can be inherited as an autosomal dominant disease. Hejazi et al. reported a heterozygous *MASP2* variant in a paediatric patient with Crohn's disease and severe tuberculosis infection (12). It has been observed that some heterozygous or homozygous variants cause significant decreases in protein levels in studies that examined the relationship between *MASP2* variants and serum or plasma enzyme levels. In addition, low enzyme levels were not observed in all individuals who carried variants of MASP2, which cause decreased enzyme levels. For example, according to the GnomAD database, the first identified variant, p.(D120G), was found frequently in the population (2.21%), indicating incomplete penetrance of this disease (11).

MASP2 protein levels are diverse among ethnic groups and populations, and variations are associated with enzyme levels (13). Low serum MASP2 levels are related to increased susceptibility to infections in several settings (14, 15). Mistegaard *et al.* identified low MASP2 levels in patients with common variable immune deficiency (CVID) and suggested that dysregulated enzyme levels may have a master or slave effect in some patients with CVID (16). In addition, *MASP2* gene variations that result in lower serum levels are associated with systemic lupus erythematosus susceptibility (17).

Here, we report a likely pathogenic heterozygous missense *MASP2* gene variant in a 6-month-old patient diagnosed with giant cell hepatitis with autoimmune haemolytic anaemia.

MATERIAL AND METHODS

Patient

A 6-month-old boy was admitted with pallor and a rapid drop in haemoglobin. He had mild jaundice and hepatosplenomegaly on admission. He was born to unrelated parents. Laboratory examination revealed direct antibody test (DAT)-positive haemolytic anaemia with mildly elevated transaminase levels (Table 1). Infectious and metabolic workup, serum immunoglobulin levels, and lymphocyte immunophenotyping were normal. Methylprednisolone 2 mg/kg/day was started. Because of early-onset autoimmune haemolytic anaemia, genetic testing was also planned to rule out underlying primary immunodeficiency. This study was approved by the İstanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 24.01.2020, No: 02). Written and oral informed consent was obtained from family members or legal representatives.

Whole-exome sequencing and data analysis

Peripheral blood samples were collected from the index case (II-1) and the parents (mother; I-2 and father I-1). DNA isolation was performed from peripheral blood using a QIAamp DNA Blood Mini kit (QIAgen Valencia, CA, USA). The amount and quality of the isolated DNA samples were evaluated using a spectrophotometry device (ND-2000, Thermo Fisher Scientific, MA, United States). Whole-exome sequencing (WES) was performed in the index case. An Agilent SureSelect Human All Exon V6 (Agilent Technologies, Santa Clara, CA, USA) kit was applied based on the manufacturer's protocol for a pre-captured library, and sequencing was performed in paired-end mode using the Illumina HiSeq 2000 platform (Illumina Inc., San Diego, CA, US). The sequence reads were mapped to the hg19 reference genome using the Burrows-Wheeler Aligner (BWA) programme (18). Aligned data were converted to VCF format for variant calling and base quality calibrations using the Genome Analysis Toolkit (GATK) (https://gatk.broadinstitute.org/hc/en-us). Variants with a Phred score of 30 (≥Q30) confidence were called. Variants were annotated using the ANNOVAR (https://annovar.openbioinformatics.org/en/latest/) and FRANKLIN (https://franklin.genoox.com/clinical-db/home) tools. Variants were categorised based on the American College of Medical Genetics and Genomics (ACMG) classification, and pathogenic (P), likely pathogenic (LP), or variant of unknown significance (VUS) variants were included. Two different analysis pipelines were applied for variant prioritisation (Figure 1). Global filtering was applied for coding and splice region variants with minor allele frequency (MAF)<0.005, P, LP, and VUS variants according to ACMG and Clinvar records were filtered. The primary immunodeficiency (PID)-related genes with a minimum MAF<0.05 and ACMG classification were filtered using a second filtering approach. The PID-specific gene list (including 451 genes) was generated according to the International Union for Immunological Societies (IUIS) guidelines and the literature knowledge (Supplemental Table 1). *In silico* prediction tools; Combined Annotation-Dependent Depletion (CADD), Sorting Intolerant From

Table 1: Clinical characteristics of the patient and the mother.

Clinical findings	Index case	Mother	Reference values
Peripheral blood tests			
WBC (10^9xL)	28.8	9.3	5.2-12.4
Hgb (g/dl)	5.8	13.5	12-18
Plt (10^3x µL)	67.3	349	130-400
HCT (%)	16	41	37-52
MCV (fl)	88.8	85.4	80-99
AST (IU/L)	4841	NA	0-40
ALT (IU/L)	3988	NA	0-40
GGT (IU/L)	106	NA	3-25
ALP (IU/L)	442	NA	20-155
Total biluribin (mg/dL)	20.18	NA	0.3-1.2
Direct biluribin (mg/dL)	17	NA	0-0.2
Direct Coombs	AHG+3, IgG+3, C3d+3	NA	Negative
PT (sec)	12.6	NA	10.4-14
APTT (sec)	12.6	NA	21-32
INR	1.07	NA	0.85-1.15
AFP (U/L)	78.63	NA	<13
CRP (mg/L)	29.39	NA	<5
Cold agglutinin (mg/L)	Positive	NA	Negative
Complement values			
C3 (mg/L)	1.54	1.04	09.-1.8
C4 (mg/dL)	0.21	0.22	Negative
Immunoglobulin levels			
IgA (mg/dL)	31.2	127	(Ref. 19)
IgG (mg/dL)	528	1255	(Ref. 19)
IgM (mg/dL)	98.3	149.8	(Ref. 19)
IgE (mg/dL)	33.95	NA	(Ref. 19)
Autoimmune antibodies			
ANCA	Negative	NA	Negative
ANA	Negative	NA	Negative

WBC: White blood cell, Hgb: hemoglobin, Plt: platelet, HCT: hematocrit, MCV: mean corpuscular volume, AST: aspartate transferase, ALT: alanine transaminase, GGT: gamma-glutamyl transferase, ALP: alkaline phosphatase, PT: prothrombin, APTT: activated partial thromboplastin time, INR: international normalized ratio, AFP: alpha-fetoprotein, CRP: c-reactive protein, C3: complement component 3, C4: complement component 4, IgA: immunoglobulin A, IgG: immunoglobulin G, IgM: immunoglobulin M, IgE: immunoglobulin E, ANCA: antineutrophil cytoplasmic antibodies, ANA: antinuclear antibody, Ref: reference

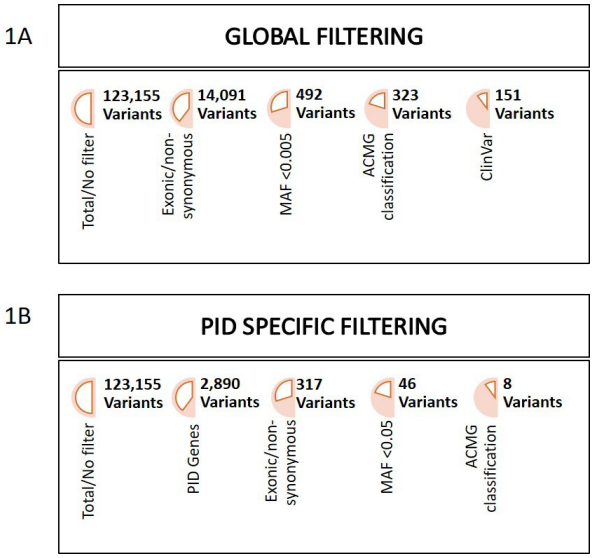

Figure 1: Variant filtering strategy for whole-exome sequencing data. **A)** Overview of global and **B)** Primary Immune Deficiency (PID)-specific variant filtering strategies with the total number of variants for each filtering step.

Tolerant (SIFT), Polymorphism Phenotyping ver.2 (PolyPhen-2), MutationTaster, and Functional Analysis through Hidden Markov Models (FATHMM), were used to predict the impact of the variants. Sanger sequencing detected the familial segregation and CLC genomics workbench 3.6.5 (QIAGEN, Aarhus, Denmark) used for analysis. Primers are available upon request.

MASP2 protein expression

Plasma samples were collected from the index case (III-5), the affected mother (II-6), and five healthy individuals with a mean age of 26 years (range: 25-34 years). MASP2 levels were determined by Enzyme-Linked Immuno-Sorbent Assay (ELISA) using a human MASP2 ELISA kit (Catalogue No: MBS2506522, MyBioSource, San Diego, USA) according to the kit's protocol. Immobilised mannan was used as a ligand to investigate the functional activity of the LP pathway. Mannan was purchased from Sigma (from Saccharomyces cerevisiae; M7504), phosphate-buffered saline (PBS) was used as solvent (10 mg/ml), and stored at 20°C. ELISA plates (microtiter plates) were coated with mannan (100 g/ml; 100 L per well) in 100 mM Na2CO3/NaHCO3 buffer (coating buffer, pH 9.6) overnight at +4°C. After each processing step, 100 µl of PBS containing 0.05% Tween-20 was used to wash the plates three times. Residual binding areas were covered by 100 µL PBS including 1% bovine serum albumin (BSA) for 1 h at 37°C. Human plasma samples diluted 1:4 using dilution buffer (kit carrier) were pre-incubated on ice for 15 min. After that, the plates were incubated at 37°C for 1 h. The minimum measurable MASP-2 concentration using the ELISA kit was 3.13 ng/mL. An enzyme-linked immunosorbent assay reader was used to measure colour intensity, and reading was performed at 450 nm. Concentration calculations were drawn as a "four-parameter logistic curve in log-log graph" paper, with standard concentration values written on the X-axis and OD values on the Y-axis. The concentration was calculated from the curve by multiplying by the dilution ratio. Comparison of MASP2 levels between the groups was performed using the Mann-Whitney U test, a non-parametric test, with GraphPad Prism version 5.01. (GraphPad Software, San Diego, California, USA, www.graphpad.com).

RESULTS

Clinical history of the index case

The patient received intravenous immunoglobulin (IVIG) at a total dose of 2 g/kg because the haemolytic anaemia did not improve. After haemoglobin levels stabilised without active haemolysis during the second month of steroid treatment, cyclosporine was added for maintenance and methylprednisolone was tapered. However, at follow-up, transaminase and bilirubin levels progressively increased (Table 1) and remained high despite discontinuation of cyclosporine and steroids. Liver biopsy was performed with a presumptive diagnosis of autoimmune or toxic hepatitis. Pathological examination revealed giant cell hepatitis. The final diagnosis was giant cell hepatitis and autoimmune haemolytic anaemia. Because the patient experienced seizures with encephalopathy and nephrotoxicity after the resumption of cyclosporine therapy, methylprednisolone was restarted. As recommended in the literature, an anti-CD20 monoclonal antibody (rituximab) at the standard dose (375 mg/m^2) was administered once weekly for six consecutive weeks in addition to steroid therapy. Eight weeks after the start of rituximab treatment, alanin aminotransferase (ALT) and aspartate aminotransferase (AST) levels returned to normal, and bilirubin levels decreased significantly to 1.3 g/dL, with normal haemoglobin levels on complete blood count (CBC) and persistence of DAT positivity (IgG 2+/C3 2+). Maintenance therapy with azathioprine was initiated, and steroid therapy was tapered and eventually discontinued. DAT became negative after two years of immunosuppressive therapy. He remained on azathioprine therapy and had no active signs of haemolysis or hepatic impairment at regular follow-up of four years after diagnosis. The father and mother had no medical history and were found to be healthy on clinical examination (Table 1) (19).

Whole-exome data analysis and *in silico* prediction

Whole-exome sequencing was performed in the index case. Global filtering was applied to the coding regions (Figure 1), MAF<0.005, nonsynonymous, and ACMG scores for the P, LP, and VUS variants (Figure 1). Filtered genes/variants (n=151) are listed in Supplemental Table 2 that were found to be irrelevant to the phenotype.

Based on the diagnosis of early-onset autoimmune haemolytic anaemia, no known pathogenic/likely pathogenic variants were found, except for the heterozygous *ABCA4*:p.(Gly172Ser) variant. Monoallelic *ABCA4* variants are associated with age-related macular degeneration (MIM:153800). The patient had no clinical symptoms at the current age, so follow-up was planned. In further analysis, the patient was screened for variants in a 451 PID-related genes list (see Supplemental Table 1), and eight candidate variants were detected (Figure 1 and Table 2). Among these, the heterozygous missense variant NM_006610.4:c.1853G>A (rs764932450) in the serine protease domain of *MASP2* was found to be relevant to clinical findings. The GnomAD frequency of the MAF was G=0.000008, ACMG class was VUS (evidence PP3 and PM2), and the aggregated prediction score was harmful. Sanger sequencing confirmed heterozygosity in the index case (III-5) and the mother (II-6) (Figure 2).

MASP2 consists of 12 exons: the serine protease (SP) domain, which is responsible for protease activity, two C1r/C1s/Uegf/bone morphogenetic protein (CUB) domains (CUB1 and CUB2), located in the heavy chain, the epidermal growth factor-like (EGF) domain, which separates the two CUB domains, and two complement control protein (CCP) domains (CCP1 and CCP2). The p.(Cys618Tyr) missense variant was found in exon 12, which encodes the serine protease domain (Figure 3A). On the 3D structure of the MASP2 protein, p.(Cys618Tyr) variant was predicted to disrupt disulphide bonds within CYS598 and CYS618 (Figure 3A). To determine the pathogenicity of the p.(Cys618Tyr) variant, SIFT, PolyPhen2, and Mutation Taster predictions were used and shown to be disease-causing, deleterious, and probably damaging, respectively. The CADD score was 24, and the variant position was also evolutionarily conserved among the species (Table 2 and Figure 3B).

Impact of the *MASP2* variant on plasma levels

To investigate the effect of the NM_006610.4:c.1853G>A;p.(Cys618Tyr) variant on active MASP2 levels, ELISA was performed on plasma samples from the index, mutated mother, and healthy controls (n=5). The MASP2 levels were 153 ng/ml in the patient and 152.3 ng/ml in the mother. The median MASP2 level in healthy control samples was 185 ng/ml (min 159-max 603 ng/ml). A decrease in MASP2 levels was found in index cases and mothers with heterozygous variation compared with healthy controls, but there was no statistical significance (p=0.12).

Table 2: The list of candidate variants after filtering based on the Primary Immune Deficiency specific filtering.

Gene	Transcript	dbSNP	AA change	Effect	Zygosity	Frequency	SIFT	PolyPhen-2	Mutation taster	CADD
ERCC4	NM_005236.3:c.2624A>G	rs1800124	p.Glu875Gly	Missense	het	G=0.013304/3345	Deleterious	Possibly damaging	D	25.08
FANCA	NM_000135.4:c.2941T>C	rs191943709	p.Cys981Arg	Missense	het	G=0.000036/9	Deleterious	Probably damaging	D	22.6
FERMT1	NM_017671.5:c.1831C>G	N/A	p.Gln611Glu	Missense	het	N/A	Tolerated	Possibly damaging	D	23.05
FERMT3	NM_031471.6:c.772G>T	rs139416960	p.Asp258Tyr	Missense	het	A=0.000012/3	Deleterious	Probably damaging	D	32
MASP2	**NM_006610.4:c.1853G>A**	**rs764932450**	**p.Cys618Tyr**	**Missense**	**het**	**G=0.000008/2**	**Deleterious**	**Probably damaging**	**D**	**24**
POLE	NM_006231.4:c.6610G>A	rs1060500871	p.Val2204Met	Missense	het	T=0.000014/2	Tolerated	Possibly damaging	D	22.8
POLE	NM_006231.4:c.6494G>A	rs5745068	p.Arg2165His	Missense	het	T=0.005893/1432	Tolerated	Possibly damaging	D	25.9
TLR3	NM_003265.3:c.1660C>T	rs121434431	p.Pro554Ser	Missense	het	T=0.000528/74	Deleterious	Probably damaging	D	24.1

Het: Heterozygous, AA: amino acid, SIFT: The Sorting Intolerant from Tolerant, PolyPhen-2: Polymorphism Phenotyping ver.2, CADD: Combined Annotation-Dependent Depletion

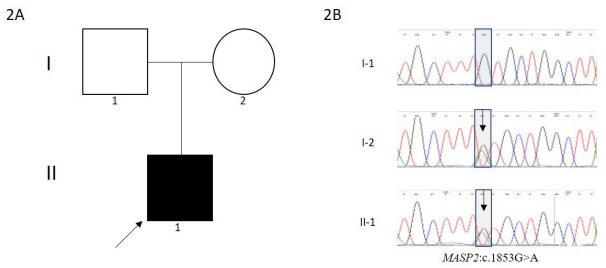

Figure 2: Family tree and segregation analysis. A) Pedigree of the *MASP2* family. B) Segregation analysis of the *MASP2* variant. The open shapes in the pedigree represent phenotypically unaffected family members, and the black square represents the affected proband. The arrows indicate the heterozgous alles of the parents.

often contributes to the pathogenesis of GCH-AHA (20, 21). Clinical findings such as hepatitis and Coombs-positive anaemia may indicate the involvement of B-cell related autoimmune dysregulation or complement deficiency. Studies have shown a high degree of complement-mediated hepatocyte injury in patients with GCH-AHA (5, 22). In this study, WES identified the heterozygous missense variant NM_006610.4:c.1853G>A in *MASP2* in a 6-month-old patient with GCH-AHA. The patient presented with hepatosplenomegaly, mildly elevated transaminase, and DAT-positive haemolytic anaemia. The pathogenic p.(Cys618Tyr) variant is located in the SP domain of the protein and is responsible for the catalytic activity of the entire molecule and may prevent the formation of the disulphide bond that binds the two polypeptide chains together in

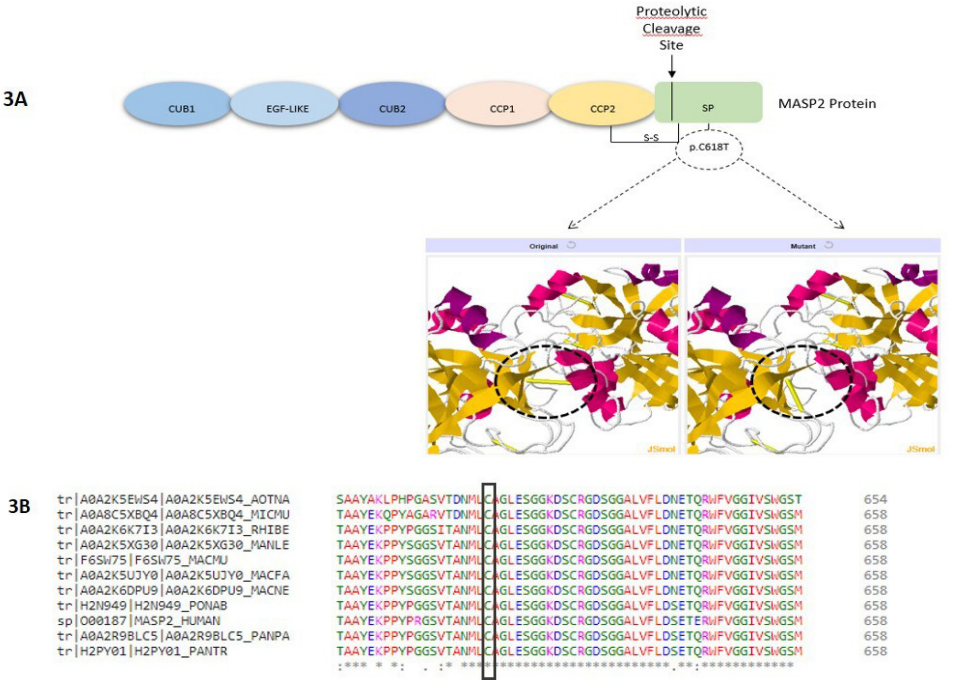

Figure 3: Localisation of p. Cys618Thr variant on the serine protease (SP) domain of MASP2 and a graphical demonstration of the wild-type and mutant forms of the MASP2 protein. 3A**)** The wild-type residue is shown to form a disulphide bond with residues CYS 598 and CYS 618 (Distance: 1.912 Å) on chain B of the wild-type structure 1q3x and the substitution disrupts this bond. 3B**)** Conservation of 618. amino acids of the MASP2 protein in primates. Conservation analysis was performed using the CLUSTAL Omega (1.2.4) multiple sequence alignment tool. The C1r/C1s/Uegf/bone morphogenetic protein 1 (CUB1) domain is an epidermal growth factor-like (EGF) domain, and the CUB2 domain is followed by two complement control protein (CCP1 and CCP2) domains and the C-terminal SP domain. The SP domain is linked to the CCP2 domain by a small linker region with S-S disulphide bonds.

DISCUSSION

Giant cell hepatitis associated with autoimmune haemolytic anaemia is associated with infection, cholestasis, and hepatic inflammation. The aetiology of this disease has not been fully elucidated, it is a factor that plays a role in immune system dysregulation. The involvement of immune-mediated findings, such as autoimmunity, most

the functionally active form of the MASP-2 protein (23). Although the mode of inheritance was autosomal dominant and segregation analysis confirmed that the mother also carried the same variation without obvious clinical symptoms, MASP2 deficiency is difficult to diagnose due to unclear genotype-phenotype correlation, low penetrance, and significant clinical heterogeneity (24).

Previous studies have shown that different mono- or bi-allelic variants of *MASP2* result in changes in serum/plasma levels and functions of MASP2 (11). Homozygous *MASP2* variants, which are believed to affect the protein, do not always cause low enzyme levels. On the contrary, some heterozygous variants can cause significant enzyme deficiency (13). The relatively low levels of MASP2 in mothers compared with healthy controls support the variable expressivity reported previously, but there is wide variability in healthy controls. Additionally, MASP2 enzyme levels were not related to age, gender, or physical activity, and there were no statistically significant differences between serum and plasma enzyme levels. Ytting et al. showed that the mean serum enzyme level in healthy controls was 416 ng/mL (ranging from 125 to 1152) and that MASP2 levels below 100 ng/mL were indicative of MASP2 deficiency in Caucasians (13, 25). To have a better understanding of the exact effects of these *MASP2* variants, the activation of the lectin pathway should be investigated in detail. GCH-AHA is a rare disorder and the genetic aetiology is unclear. Possible mechanisms include autoimmunity or dysregulation of the complement system affecting hepatocytes and erythrocytes. *MASP2* variants may contribute to the pathogenesis of GCH-AHA as a primary or secondary disease. Evaluation of enzyme levels and MBL pathway activity is the most accurate approach to assessing the clinical impact of monoallelic or biallelic *MASP2* variants.

CONCLUSION

This study presented the clinical findings and genetic analysis of an index case diagnosed with giant cell hepatitis and autoimmune haemolytic anaemia. Despite initial treatment challenges and complications, including progressive elevation of transaminase and bilirubin levels following cyclosporine therapy and subsequent seizures with encephalopathy and nephrotoxicity, our patient experienced significant improvement following a comprehensive therapeutic approach. Whole-exome sequencing revealed a heterozygous missense variant NM_006610.4:c.1853G>A (rs764932450) in the serine protease domain of MASP2, which was deemed relevant to the clinical phenotype. Through *in silico* predictions and functional assays, we elucidated the potential pathogenicity of the identified variant and its contribution to the observed phenotype.

Ethics Committee Approval: The study has ethical approval from the İstanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 24.01.2020, No: 02).

Informed Consent: Written and oral informed consent was obtained from family members or legal representatives.

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REFERENCES

1. Perez-Atayde AR, Sirlin SM, Jonas M. Coombs-positive autoimmune hemolytic anemia and post infantile giant cell hepatitis in children. Pediatr Pathol 1994;14(1):69-77. [CrossRef]
2. Bakula A, Socha P, Klaudel-Dreszler M, Karolczyk G, Wozniak M, Rutynowska-Pronicka O, et al. Giant cell hepatitis with autoimmune hemolytic anemia in children: proposal for therapeutic approach. J Pediatr Gastroenterol Nutr 2014;58(5):669-73. [CrossRef]
3. Maggiore G, Sciveres M, Fabre M, Gori L, Pacifico L, Resti M, et al. Giant cell hepatitis with autoimmune hemolytic anemia in early childhood: long-term outcome in 16 children. J Pediatr 2011;159(1):127-32. [CrossRef]
4. Torbenson M, Hart J, Westerhoff M, Azzam RK, Elgendi A, Mziray-Andrew HC, et al. Neonatal giant cell hepatitis: histological and etiological findings. Am J Surg Pathol 2010;34(10):1498-503. [CrossRef]
5. Nastasio S, Matarazzo L, Sciveres M, Maggiore G. Giant cell hepatitis associated with autoimmune hemolytic anemia: an update. Transl Gastroenterol Hepatol 2021;6:25. [CrossRef]
6. Whitington PF, Vos MB, Bass LM, Melin-Aldana H, Romero R, Roy CC, et al. Humoral immune mechanism of liver injury in giant cell hepatitis with autoimmune hemolytic anemia. J Pediatr Gastroenterol Nutr 2014;58(1):74-80. [CrossRef]
7. Dunkelberger JR, Song WC. Complement and its role in innate and adaptive immune responses. Cell Res 2010;20(1):34-50. [CrossRef]
8. Vorup-Jensen T, Petersen SV, Hansen AG, Poulsen K, Schwaeble W, Sim RB, et al. Distinct pathways of mannan-binding lectin (MBL)- and C1-complex autoactivation revealed by reconstitution of MBL with recombinant MBL-associated serine protease-2. J Immunol 2000;165(4):2093-100. [CrossRef]
9. Dobo J, Kocsis A, Gal P. Be on Target: Strategies of Targeting Alternative and Lectin Pathway Components in Complement-Mediated Diseases. Front Immunol 2018;9:1851. [CrossRef]
10. Tangye SG, Al-Herz W, Bousfiha A, Cunningham-Rundles C, Franco JL, Holland SM, et al. Human Inborn Errors of Immunity: 2022 Update on the Classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol 2022;42(7):1473-507. [CrossRef]
11. Stengaard-Pedersen K, Thiel S, Gadjeva M, Moller-Kristensen M, Sorensen R, Jensen LT, et al. Inherited deficiency of mannan-binding lectin-associated serine protease 2. N Engl J Med 2003;349(6):554-60. [CrossRef]

12. Hejazi R, Hasosah M. Tuberculosis, onychomycosis and immune deficiency in complicated Crohn's disease. BMJ Case Rep 2019;12(8):e228986. [CrossRef]
13. Thiel S, Steffensen R, Christensen IJ, Ip WK, Lau YL, Reason IJ, et al. Deficiency of mannan-binding lectin associated serine protease-2 due to missense polymorphisms. Genes Immun 2007;8(2):154-63. [CrossRef]
14. Boldt AB, Luz PR, Messias-Reason IJ. MASP2 haplotypes are associated with high risk of cardiomyopathy in chronic Chagas disease. Clin Immunol 2011;140(1):63-70. [CrossRef]
15. de Rooij BJ, van Hoek B, ten Hove WR, Roos A, Bouwman LH, Schaapherder AF, et al. Lectin complement pathway gene profile of donor and recipient determine the risk of bacterial infections after orthotopic liver transplantation. Hepatology 2010;52(3):1100-10. [CrossRef]
16. Mistegaard CE, Jensen L, Christiansen M, Bjerre M, Jensen JMB, Thiel S. Low levels of the innate immune system proteins MASP-2 and MAp44 in patients with common variable immunodeficiency. Scand J Immunol 2022;96(3):e13196. [CrossRef]
17. Xu WD, Liu XY, Su LC, Huang AF. Association of MASP2 levels and MASP2 gene polymorphisms with systemic lupus erythematosus. J Cell Mol Med 2020;24(18):10432-43. [CrossRef]
18. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 2009;25(14):1754-60. [CrossRef]
19. Besci O, Baser D, Ogulur I, Berberoglu AC, Kiykim A, Besci T, et al. Reference values for T and B lymphocyte subpopulations in Turkish children and adults. Turk J Med Sci 2021;51(4):1814-24. [CrossRef]
20. Poddighe D, Madiyeva A, Talipova D, Umirbekova B. Infantile giant cell hepatitis with autoimmune hemolytic anemia. World J Hepatol 2021;13(4):411-20. [CrossRef]
21. Unal S, Kuskonmaz B, Balamtekin N, Baysoy G, Aytac Elmas S, Orhan D, et al. Autoimmune hemolytic anemia and giant cell hepatitis: Report of three infants. Turk J Haematol 2010;27(4):308-13. [CrossRef]
22. Dubruc E, Nadaud B, Ruchelli E, Heissat S, Baruteau J, Broue P, et al. Relevance of C5b9 immunostaining in the diagnosis of neonatal hemochromatosis. Pediatr Res 2017;81(5):712-21. [CrossRef]
23. Ambrus G, Gal P, Kojima M, Szilagyi K, Balczer J, Antal J, et al. Natural substrates and inhibitors of mannan-binding lectin-associated serine protease-1 and -2: a study on recombinant catalytic fragments. J Immunol 2003;170(3):1374-82. [CrossRef]
24. Garcia-Laorden MI, Hernandez-Brito E, Munoz-Almagro C, Pavlovic-Nesic S, Rua-Figueroa I, Briones ML, et al. Should MASP-2 Deficiency Be Considered a Primary Immunodeficiency? Relevance of the Lectin Pathway. J Clin Immunol 2020;40(1):203-10. [CrossRef]
25. Ytting H, Christensen IJ, Thiel S, Jensenius JC, Svendsen MN, Nielsen L, et al. Biological variation in circulating levels of mannan-binding lectin (MBL) and MBL-associated serine protease-2 and the influence of age, gender and physical exercise. Scand J Immunol 2007;66(4):458-64. [CrossRef]