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Biallelic Variant in *SERPING1* Associated with Intellectual Disability in a Consanguineous Pakistani Family

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Intellectual disability, Synaptic refining, Autosomal recessive, Whole exome sequencing, C1INH, Hypotonia

ABSTRACT

The complement system is part of humoral, innate humor immune system, which enhance the ability of antibodies and phagocyte cells to clear microbes and damage cells from an organism. It plays a crucial role in brain development, neurogenesis, neural migration, and synaptic refining. Pathogenic variants in genes, including *SERPING1*, of complementary pathways are associated with psychosis and schizophrenia. The *SERPING1* encodes highly glycosylated serine protease inhibitor C1INH, which inhibits proteins of both the classical lectin pathways of the complement system and protein that plays a key role in the complement system pathway. Here, we report a biallelic missense variant (c.5 C>T; p.(Ala2Val)) in *SERPING1* segregating with severe intellectual disability (ID), hypotonia, psychomotor and speech delay in consanguineous Pakistani kindred. The p.(Ala2Val) variant is predicted pathogenic by various in silico algorithms and replaces an evolutionary conserved residue. *SERPING1* is known to interact with *PTX3, MASP1,* and *C1QBP* genes, variants of which have been associated with ID in humans. Our study expands the genetic repertoire of variants responsible of ID in the Pakistani population.

INTRODUCTION

The role of immunological and inflammatory factors has been associated with psychosis and schizophrenia (Goldsmith *et al*., 2016). The complement system, a crucial component of the inflammatory response, has also been extensively studied in this context (Kopczynska *et al*., 2019). The complement system is the cascade of soluble proteins that are released by a number of cell types including, hepatocytes, leucocytes, adipocytes, and neural cells (neurons, astrocytes, and microglia) in the central nervous system (Bajic *et al*., 2015; Veerhuis *et al*., 2011). These soluble proteins and zymogens recognize the pathogen and extrinsic antigens and initiate opsonization, pathogen phagocytosis, and anaphylatoxin activity (Bajic *et al*., 2015; Ricklin *et al*., 2010). The system is classified into three separate pathways- classical, lectin, and alternative- depending on their mode of activation. Curiously, all the pathways converge at factor C3 cleavage and complement effector fragments. The system is strictly regulated and specified to protect the host cells from autoimmune response (Coulthard *et al*., 2018). Evidently, all components of the complement system are expressed in the brain and play a key role in the brain development, neural migration (Gorelik *et al*., 2017), neurogenesis (Coulthard *et al*., 2017; Rahpeymai *et al*., 2006) and synaptic refining (Schafer *et al*., 2012; Stephan *et al*., 2012).

Genomic data-based analysis of case vs control study

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of 1000 psychosis individuals show *SERPING1* variant harboring individuals have reduced intelligence quotient as compared to other 90 genes associated with psychosis (Holland *et al*., 2019). The *SERPING1* encodes highly glycosylated serine protease enzyme C1INH (C1 inhibitor) that inhibits C1r and C1s, the initial components of the classical complement system pathway. C1INH is known to interact with and modulate the activity of *MASP1/MASP2, a* protein involved in the lectin pathway for complement activation (Paréj *et al*., 2013; Presanis *et al*., 2004; Ricklin *et al*., 2010). Here, we present evidence of a biallelic missense mutation in *SERPING1* that segregates with severe intellectual disability (ID), hypotonia, and delayed speech in a Pakistani consanguineous family.

MATERIALS AND METHODS

Family PKMR442 was enrolled from Multan city of Punjab province of Pakistan, after obtaining written informed consent from all the participants. Affected individuals underwent examinations by a neurologist and primary physician to assess specific features associated with ID, behavior, neurological and motor delays, ophthalmological, auditory, and skeletal abnormalities.

Table I. ES analysis of family PKMR 442.

Exome sequencing data	PKMR222 individual IV-1
Total mapped read (Gbp)	5-6
Target coverage (%)	96-97
Mean depth of coverage (x)	65-80
Depth >20 x coverage (%)	80-90
Total changes	285384
Non-synonyms/insertion/deletion/splice site	57489
Changes <0.01% allele frequency in 1000 genome/NHLBI exome/ExAC database	20171
Changes not found in Pakistani control sample populations	12009
Genes with homozygous/compound heterozygous changes	301
Missense/indels/splice variants	59
Changes predicted to be pathogenic	11
Gene with nucleotide variation in more than one affected individual	1
Changes co segregating with the phenotype in the family	1

Blood samples were collected for DNA extraction using the inorganic method (MWer *et al*., 1988). ES (exome sequencing) was performed on the DNA sample of the proband (IV:3) at the University of Maryland and the *SERPING1* variant was identified by using exome filtering criteria as described previously (Usmani *et al*., 2024). Furthermore, Sanger sequencing was performed using gene-specific primers to confirm the co-segregation of the *SERPING1* variant with the ID phenotype in the family. *In silico* analyses were performed using the Varsome, SMART, Alphafold2 Clustal Omega, and Allen Brain Atlas.

RESULTS

Clinical data

According to the medical evaluation, all three affected individuals (IV:1, IV:3 & IV:7) of PKMR442 (Fig. 1A, B) have severe ID (HP:0010864), hypotonia (HP:0001252), show psychomotor (HP: 0001263) and speech delay (HP:000750) except for subject IV:7 who had no speech. Behavioral analysis presented aggressive behavior (HP: 0000718) in all the affected individuals particularly IV:3, where it was extended to self-mutilation tendency. Physical examination did not show any abnormality in the siblings IV:3 and IV:7 however, individual IV-1 had abnormal stature and bone structure of the forelimbs (Fig. 1C). No visual or hearing deficits were noted in all three affected individuals (Table II). It is also notified that no sign of angioedema was observed in the siblings of the family.

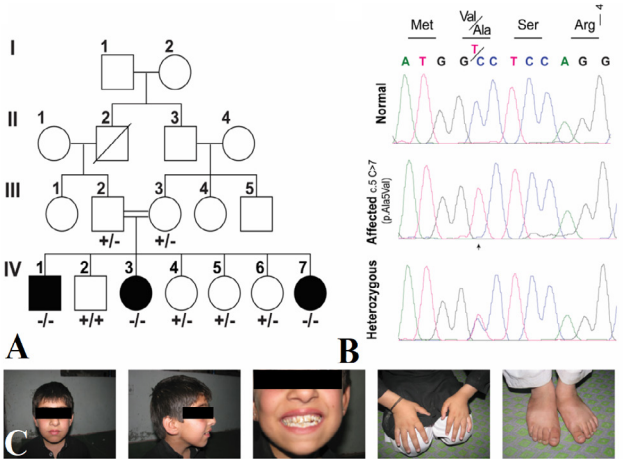

Fig. 1. A homozygous variant in *SERPING1* segregates in PKMR 422 (A) Family pedigree of PKMR 422. Square: male, circle: female, filled symbol: affected individual, the double line between individual: consanguineous mating. + symbol: Wildtype allele, - symbol: Mutant allele (B) Gene sequence chromatograph showing the nucleotide change c. 5C>T in homozygous affected and heterozygous carriers in comparison to homozygous normal alleles. (C) Phenotypic features of the affected proband individual IV-1.

Table II. Clinical and genetical features of the family PKMR 442.

Clinical and genetical features	PKMR 442	PKMR 442	PKMR 442
Individual	IV-1	IV-3	IV-7
Gender	Male	Female	Female
Age	10	19	14
Ancestry	Pakistani		
RefSeq ID	NM_001032295		
Variation	c.5C>T p. (Ala2Val)	c. 5C>T p. (Ala2Val)	c.5C>T p. (Ala2Val)
Zygosity	Homozygous	Homozygous	Homozygous
Growth features			
Weight (kg)	52	68	60
Height (cm)	138	161	145
Head circumference (cm)	55.5	57	54
Neurological features			
ID	Severe	Severe	Severe
Epilepsy	Yes	No	Yes
Ataxia	Yes	Yes	No
Hypotonia	Yes	Yes	Yes
Spasticity	No	No	No
Intellectual and neurological delay			
Speech delay	Yes	Yes	No
Psychomotor delay	Yes	Yes, Sluggish	Yes
Other features/ investigations/ angioedema	Spine, rib cage and arms bones are abnormal	No	No

Table III. Short listed variants from exome data.

S. No.	Chr	Position	Ref	Alt	Func. ref gene	Gene. ref gene	ExonicFunc. ref gene	esp6500si	ExAC_ALL
1	1	2.49E+08	G	A	Exonic	OR2T3	Nonsynonymous SNV	.	0.0042
3	2	1.31E+08	T	A	Exonic	POTEI	Nonsynonymous SNV	.	0
4	4	1016071	G	A	Exonic	FGFRL1	Nonsynonymous SNV	.	0.0001
5	6	32489883	G	T	Exonic	HLA-DRB5	Nonsynonymous SNV	.	0.0002
6	6	32489940	G	A	Exonic	HLA-DRB5	Stopgain	.	0.0003
7	11	57365748	C	T	Exonic	SERPING1	Nonsynonymous SNV	0.000787	0.0025
8	11	76922381	G	A	Exonic	MYO7A	Nonsynonymous SNV	.	0.0001
9	12	32137067	A	G	Exonic	KIAA1551	Nonsynonymous SNV	0.001999	0.0058
10	15	41798189	C	T	Exonic	LTK	Nonsynonymous SNV	0.000154	0.0001
11	16	69876022	C	T	Exonic	WWP2	Nonsynonymous SNV	.	0.0002
12	17	30980871	T	A	Exonic	MYO1D	Nonsynonymous SNV	0.002307	0.0025

Variant identification and molecular modeling

A homozygous missense variant c.5 C>T p.(Ala2Val) in *SERPING1* (NM_ 001032295) gene was identified in proband IV:3 through ES (Table III). A total of 11 gene variations (SNV) were short-listed after the analysis of the exome-sequenced data (Table III). Sanger sequencing confirmed homozygosity of only *SERPING1* variant in all three affected individuals, while parents were obligatory carriers (Fig. 1). Unaffected siblings IV:4, IV:5, and IV:6 were also heterozygous while individual IV:2 was homozygous wild type (Fig. 1).

Table IV. *In Silico* single nucleotide variation functional prediction of *SERPING1*.

In silico SNV analysis	Findings
Hg19 coordinates	Chr11:57365748
Nucleotide variation	c.5C>T
Amino acid variation	p. (Ala2Val)
RefSeq	NM_001032295
ExAC frequency	0.00125
gnomAD frequency	0000894
CADD phred	24.1
REVEL	634
M-CAP	Damaging
PolyPhen-2 HumDiv	Possibly damaging
Polyphen-2 Hum Var	Possibly damaging
GERP ++	1.84
PhyloP 1000 way vertebrate	1.57
Mutation assessor_pred	Uncertain
MutationTaster_pred	Polymorphism
FATHMM_pred	Damaging

The c.5C>T had very low allele frequency in the gnomAD database with a CADD score of 24.1 (Table IV). According to C-MAP and Polyphen-2, the variation could possibly be damaging in nature. According to the Mutation Taster *SERPING1* protein, containing the identified variation is predicted to be polymorph. While according to FATHMM the variation is predicted to be damaging to the protein with a -2.44 score (Table IV).

Serpin Peptidase inhibitor, clade G, member 1 (*SERPING1*) encodes protease inhibitory protein C1INH. The gene is located on the long arm of chromosome 11 (11q12.1), comprising 8 exons as represented by grey shaded boxes in the schematic model diagram (Fig. 2A). SMART predicted the C1 inhibitor (C1INH) protein to contain a 22-amino-acid (AA)-long signal peptide followed by a single structured domain called SERPIN depicted in blue/grey from 136 to 498 AA. The area of complexity is also shown in pink (Fig. 2B). The p.(Ala2Val) variation is situated in the signal peptide of the encoded protein, which is indicated via an arrow in the illustration. The sole functionally defined SERPIN domain uses its reactive centre loop (RCL) to interact with the target proteins of the complement system (Fig. 2B). The evolutionary conservation of the amino acid in different species was confirmed by performing multiple amino acid sequence alignments of orthologous proteins through Clustal Omega (Fig. 2C). AlphaFold 2 confirmed the mutated region to lie in the non-structural region of the protein with a pLDDT score of 35.5 (Fig. 2E); however, the missense alpha heatmap predicts the p.(Ala2Val) to be likely pathogenic (having a 0.72 score).

We researched the available literature and used "STRING" tool to extract all reported protein-protein interactions and noted the direct interaction of *SERPING1* with *PTX3, MASP1,* and *C1QBP* (Fig. 2D) (Szklarczyk *et al*., 2023). Variants of *PTX3, MASP1*, and *C1QBP* genes have been previously reported in individuals with ID (Feichtinger *et al*., 2017; Rooryck *et al*., 2011). Our analysis from Allen Brain Atlas also shows a high expression of SERPING1 in the human developing brain, which overlaps with these known interactors.

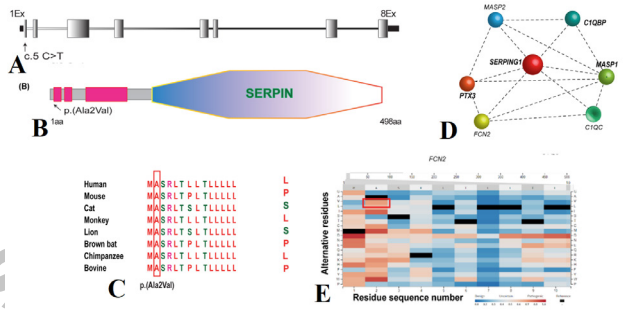

Fig. 2. Molecular Structure Modeling of *SERPINGI* (A) Schematic representation of human *SERPINGI* gene including the position of the nucleotide change. The grey shaded boxes represent exons of the gene. (B) Schematic representation of human *SERPINGI* protein with the predicted effect of the identified variant in the study showing SERPIN domain in blue and area of complexity in pink. (C) Clustal W amino acid sequence alignment of the *SERPINGI* from human, mouse, cat, monkey, lion, brown bat, chimpanzee, and bovine. The square orange box indicates the position of the mutated amino acid conserved across the vertebrates. (D) STRING analysis based on SERPINGI protein interaction with various proteins in nature, the highlighted genes PTX3, MASP1, and CIQBP are associated with ID-disorder in Humans. (E) AlphaMissense Pathogenicity Heatmap showing mutated residue p.(Ala2Val) to be likely pathogenic presented in a red colored box with the score of 0.722.

DISCUSSION

ID refers to an individual's limitation in cognitive functioning and adaptive behavior. It is a phenotypically and genetically heterogeneous disorder with a prevalence of approximately 2.5% worldwide (Maulik *et al*., 2011). Here, we demonstrate the role of ES approach in identifying pathogenic variant in PKMR442 family segregating ID in an autosomal recessive fashion. The

results of our studies lead us to a biallelic missense variant c.5C>T in the *SERPING1* gene which encodes C1INH with a key regulatory role in the complement system. The system is extensively studied as it is the main component of the innate immune response. The complement system is also associated with neurodevelopmental disorders, especially concerning neuronal pruning. In murine, the proteins of these pathways including C1q and C3 are localized to developing CNS synapses during periods of active synapse elimination and are required for normal brain wiring. Their functions in the brain seem to be analogous to that of the immune system involving opsonization. The synapse tagged with complement protein is eliminated by microglial cells with complement surface receptors (Stevens *et al*., 2007). Astrocytes also induce the expression of complement proteins in the brain. In a mature brain, the loss of synapses is the trademark of several neurodegenerative disorders. Upregulation of complement pathways is suggested to play a role in neural pruning and consequent neurodevelopment disorder (Holland *et al*., 2019).

Previously, *SERPING1* encoding C1-INH has been suggested to play a role in neuronal stem cell proliferation and activation of all three complement pathways (Gorelik *et al*., 2017). Knockout of *SERPING1* induces neurovascular impairment, glial cell activation, neuroinflammation, and behavioral deficits (Farfara *et al*., 2019). *SERPING1* role is suggested in neuronal migration and brain development (Gorelik *et al*., 2017). In a human genetic study schizophrenic (SZ) twin, showed involvement of *SERPING1* with cortical thinning of superior frontal region (Allswede *et al*., 2018). Another study on SZ found expression of *SERPING1* in the amygdala (Chang *et al*., 2017). Conversely, above two studies were performed in individuals with SZ, however, it is known that overall, 31.7 % of identified ID people had some sort of psychiatric disorder (Morgan *et al*., 2008). Thus, making these studies important in our quest to understand the role of *SERPING1* in ID.

In vivo, studies on *SERPING1* attenuated acute neurobehavioral and degeneration deficit along with ischemic volume (Longhi *et al*., 2009). In the gene-enriched studies of the complement pathway, out of all the gene-set, *SERPING1* is reported to be most associated with the human IQ (Holland *et al*., 2019) which supports the pivotal role of *SERPING1* in neural development and neuroinflammatory response.

CONCLUSION

A current study identified a homozygous missense variant in *SERPING1* in a consanguineous Pakistani family PKMR 442 with affected siblings presenting with autosomal recessive ID, hypotonia, and speech delay.

The results of our findings are consistent with the studies that associate *SERPING1* with neurodevelopmental and cognitive functioning making it a candidate gene for elucidation of ID etiology. Hence, making it a key regulator of the complement system that inhibits the inappropriate activation of neural pruning and neuroinflammation response.

DECLARATIONS

Acknowledgment

This study received approval from the Institutional Review Boards at the Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan, and University of Maryland, Baltimore, USA. We would like to thank the participating individuals and their families and health care professionals involved in their care.

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IRB statement

The study was conducted and approved by the Institutional Review Board (IRB) of the Center of Excellence in Molecular Biology, Lahore, Pakistan, and the University of Maryland, Baltimore, USA.

Ethical statement

This study was approved by the ethics committee of Centre of Excellence in Molecular Biology, University of the Punjab Lahore (No: IRB-ID/2023), and the informed consent was obtained from guardian of the patients and all participants.

Informed consent statement

Informed consent was obtained from all the individuals involved in the study.

Statement of conflict of interest

The authors have declared no conflict of interest.

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