Original Article

A Missense Variant in LAMA3 Gene Causes Microcephaly and Epidermolysis Bullosa in a Pakistani Family

Zaib-Un-Nisa Mughal, Jawaid Ahmed Zai, Muhammad Ansar

ABSTRACT

OBJECTIVE: To identify the disease-causing mutation in a family with autosomal recessive primary microcephaly (MCPH).

METHODOLOGY: This cross-sectional study was the continuation of an ongoing family-based study initiated in 2016 at the Department of Biochemistry, Quaid-e-Azam University, Islamabad. The family was selected randomly and recruited from Sahiwal and has three members with MCPH. DNA was isolated from blood samples and the genome-wide scan was performed to map homozygous regions. Whole exome sequencing (WES) was performed to identify the plausible gene variant.

RESULTS: Whole genome data analysis identified multiple homozygous regions, but none of these contain known MCPH genes. Whole exome sequencing (WES) data identified six potentially pathogenic variants but only the Laminin subunit alpha-3 (LAMA3) (c.5260C/T) variant segregates in the family and is also present within the genomic region mapped on chromosome 18. The reevaluation of affected members of the family revealed the presence of blisters on their hands and feet indicating the presence of epidermolysis bullosa along with microcephaly.

CONCLUSION: The casual finding of the LAMA3 variant (c.5260C/T; p. Arg1754Trp) and absence of any other MCPH causing variant in affected members of this family expands the phenotypic spectrum of LAMA3 associated phenotype. Therefore, we can conclude that the LAMA3 variant can probably cause recessive microcephaly and epidermolysis bullosa, but additional studies are needed to establish the role of LAMA3 in microcephaly.

KEY WORDS: MCPH, LAMA3, Laminin-5, Genome scan, homozygous regions, whole-exome sequencing.

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INTRODUCTION

Microcephaly is a type of neurodevelopmental disorder that is frequently present in patients with intellectual disabilities (ID). Microcephaly is characterized by reduced cranial volume (below the 3 standard deviations at birth) and mild to severe ID but additional neurological deformities are absent in the patients[1]. The reduction in the cranial volume of the newborns and children can be detected by measuring occipital–frontal circumferences (OFC), which is significantly reduced in microcephalic cases. Detailed neurological imaging studies of microcephalic patients have shown a decrease in the cerebral cortex which leads to a simplified gyral patterning without affecting the cerebral cortex thickness[2, 3]. To date, twenty-five genes have been identified that cause autosomal recessive primary microcephaly (MCPH)[3] and among these 12 genes (*MCPH1*[4], *WDR62*[5], *CDK5RAP2*[6], *ASPM*[7], *CENPJ*[6], *CEP135*[8], *CEP152*[9], *CDK6*[10], *SASS6*[11], *MFSD2A*[12], *KIF14*[13], and *NUP37*[14]) have been identified by studying Pakistani families. Previous studies on Pakistani and other populations have identified *ASPM* and *WDR62* genes as major players responsible for MCPH[1, 2].

The Laminin-5 protein (laminin-332) is a large glycoprotein mainly expressed in skin and comprises of three subunits α3 chain, β3 chain, and γ2 chains which are encoded by *LAMA3*, *LAMB3*, *LAMC3* genesrespectively[15]. This protein plays a vital role in the adhesion of the epidermal and dermal layer[16]. The *LAMA3* gene located on chromosome 18q11.2 consists of 75 exons and encodes a protein with 3333 amino acids[17]. The mutation in the *LAMA3* gene causes skin disease Herlitz type junctional epidermolysis bullosa (JEB) with recessive trait[18, 19] and Laryngol-onycho-cutaneous syndrome[20].

In this study, we performed a genetic analysis of a family segregating autosomal recessive primary microcephaly and identified a missense mutation in the *LAMA3* gene.

METHODOLOGY

The cross-sectional study was performed at the Biochemistry Department of Quaid-I-Azam University, Islamabad from 2016-2018 after getting approval by the Bio-Ethical Committee (BEC-FBS-QAU-59/2016), of Quaid-i-Azam University (QAU). The randomly selected two-generation family segregating microcephaly was recruited from a remote village of Sahiwal district from Punjab province. Written informed consent was obtained from all the subjects and their parents according to the instruction specified

in the Declaration of Helsinki. A detailed interview was conducted with elders of the family to collect information about pedigree, patient's behavior, disease status, and degree of disease progression. The occipital–frontal circumferences (OFC) of all available family members were also measured.

Peripheral blood samples were collected in EDTA (Ethylene diamine tetra-acetic acid) tubes (BD, Franklin Lakes, NJ, USA) from available family members. Genomic DNA was extracted by standard organic phenol-chloroform method, quantified by using ColibriMicrovolume Spectrometer (Titertek Berthold, Germany). After DNA quantification samples were diluted to 50ng/ul and used for genome-wide genotyping and exome sequencing in an international collaborator laboratory at Center for Statistical Genetics, Columbia University, New York, USA. Infinium® HumanCoreExomeBeadChip (Illumina, USA) was used for genome-wide scan and the resulting genotype data was analyzed by homozygosity mapper (Seelow et al., 2009).

The whole-exome sequencing (WES) of one affected individual was performed by using NimbleGenSeqCap EZ Human Exome Library v.2 (Roche Diagnostics, San Francisco, CA). The variants identified by WES were further filtered to enrich potentially pathogenic and rare variants. Selected potentially pathogenic variants were further tested for segregation in the available family members by using Sanger sequencing. Each candidate gene variant was amplified from DNA samples of available family members by using standard PCR protocol and the PCR product was purified by Gene JETTM PCR Purification Kit (Fermentas, London, UK). Sanger sequencing was performed with Big Dye Terminator cycling sequencing kit v. 3.1(Applied Biosystems, Foster City, USA) and the products were analyzed on ABI Genetic Analyzer (ABI, USA). The data was analyzed on Bioedit software version 7.0.9.0.

For the prediction of the pathogenic nature of identified variants, a public database like Mutation Taster (http://www.mutationtaster.org/), SIFT (https://sift.bii.a-star.edu.sg), polyphen2 (http://genetics.bwh.harvard.edu/pph2), and PROVEAN (http://provean.jcvi.org/) were used.

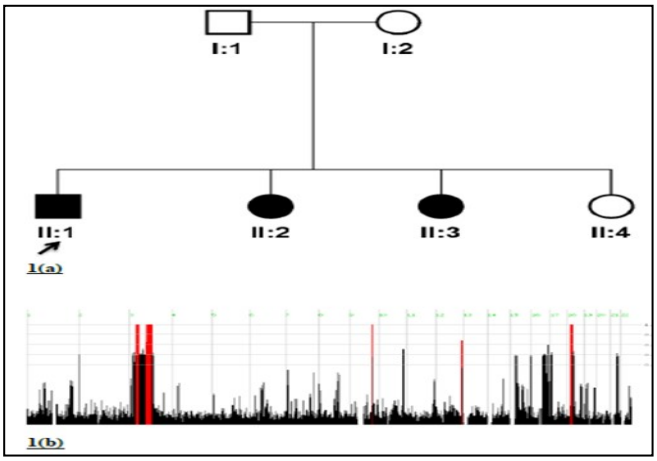
The frequency of these variants was tested with 1000 Genomes Browser (http://www.1000genomes.org), genomAD (https://gnomad.broadinstitute.org), and dbSNP (http://www.ncbi.nlm.nih.gov/SNP/).

RESULTS

The two-generation family (Figure I(a)) resides in Sahiwal district, Punjab province comprising of three affected including one male (II-1) and two females (II-2, II-3) individuals. The ages of three patients range from 9 to 14 years but all presented reduced head circumferences and severe ID. Both parents were unable to explain the exact ancestral relationship however, their elders were certain about a convergence of ancestry three generations earlier, therefore we concluded an autosomal recessive inheritance of MCPH in this family.

Analysis of the genome-wide genotyping data identified homozygous regions on chromosomes 3, 9, and 18 (Figure I(b)). The two larger 25 Mb and 11.7 Mb homozygous by descent (HBD) regions were mapped on chromosome 3 and we anticipated that MCPH causing gene may be present within any one of these regions. Further analysis of candidate genes located within these genomic regions could not identify any known MCPH gene. Therefore, one affected individual underwent for WES to identify disease-causing variants in this family.

Figure I(a): Family pedigree showing three MCPH patients in the 2nd generation. The black arrow points to the individual (II: I) which was utilized for WES (I(b)) HBD regions mapped on the respective chromosomes are shown by vertical red lines. The green row on X-axis indicates the chromosome numbers while the homozygosity score is presented on Y-axis.



The exome data analysis detected 79,637 sequence variants in affected individual 2-1. These pathogenic variants were scrutinized with different prediction tools which resulted in the shortlisting of six rare detrimental coding variants in *LAMA3* (c.5260C/T), *PIK3C2B* (c.3038G/C), *DYRK3* (c.982C/T), *GADL1* (c.1097A/G), *RGMA* (c.886C/T) and *PVRL3* (c.1492G/C) gene (Table I).

Sanger sequencing also confirmed the segregation of the *LAMA3* variant (c.5260C/T; p. Arg1754Trp) in this family (Figure II(A)). The affected individual is homozygous for the mutant allele whereas the parent is heterozygous for both alleles. This identified variant is also found within the homozygous genomic region mapped in the family by the genome-wide scan. The *LAMA3* variant (c.5260C/T; p. Arg1754Trp) is predicted as damaging by mutation taster. SIFT and PolyPhen2 also predicted this variant as deleterious. The variant is reported in the heterozygous form

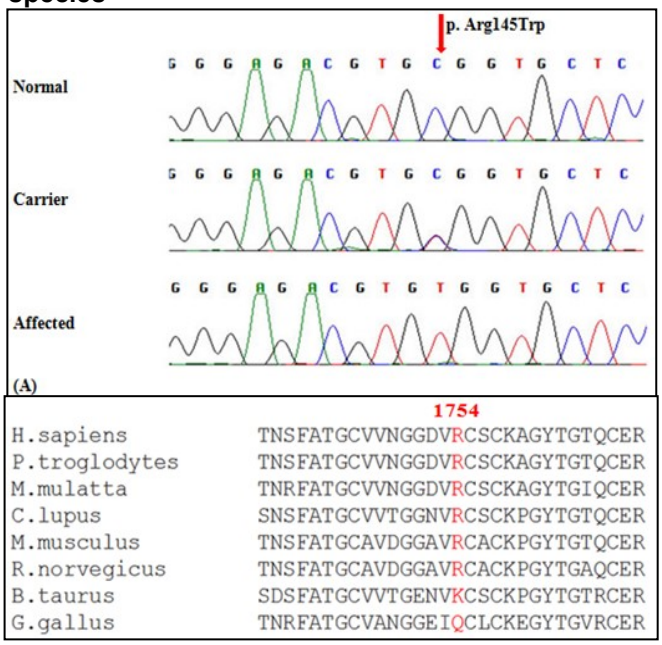
(6/121216) and (13/282308) in ExAC and gnomAD databases, respectively. Conservation analysis exhibited that the arginine amino acid residue at 1754 was almost in orthologue of higher vertebrates (Figure II (B)).

TABLE I: VARIANTS IDENTIFIED BY WES IN THE FAMILY WITH MICROCEPHALY

Gene	chr	Ref Seq Mutation	Protein change	gnomAD MAF south Asian Allele	gnomAD All MAF	1000g	MT	PP2	SIFT
LAMA3	18	c.5260C/T	p.R1754W	0.000098	0.00004	-	DC	PD	Del
GADL1	3	c.1097A/G	p. Q366R	0.00003	0.000003	-	DC	PD	Del
RGMA	15	c.886C/T	p. R296C	0.00006	0.00001	-	DC	PD	Del
DYRK3	1	c.982C/T	p. R328C	0.00019	0.00009	-	DC	B	Del
PIK3C2B	1	c.3122G/C	p. S1041T	0.000217	0.00169	-	P	PD	T
PVRL3	3	c.1492G/C	p.V498L	0.00016	0.00006	-	DC	B	T

MT= Mutation Tester, 1000g= 1000 genome, chr= chromosome, MAF= Minor allele frequency, PD= Probably Damaging, DC= Disease Causing, Del= Deleterious, SIFT= sorting intolerant from tolerant, PP2=polyphen2.

Figure II(A) Chromatogram shows the segregation of LAMA3 gene variant (c.5260C/T) in the family (B) Multiple alignments in the orthologues of LAMA3 gene showing Arginine 1754 amino acid residue is conserved among some vertebrate species



DISCUSSION

In the present study, we found an a*LAMA3* gene variant (c.5260C/T; p. Arg1754Trp) in all affected members of a family with autosomal recessive microcephaly. Laminin subunit alpha-3 (*LAMA3*) gene comprised of 75 exons that encode α3 subunit for laminin-5 protein. Laminin-5 protein is a highly complex glycoprotein that is extensively secreted in the skin and other epithelial structures where it serves as anchorage with the extracellular material and is also thought to involve in cellular migration, tissue binding, and organization in embryo[16,21,22]. *LAMA3* variants were found to be associated with a skin disorder, autosomal recessive, that results in blister formation[19]. Mouse with a targeted mutation in *LAMA3* genes exhibits severe epithelial abnormalities. Malformation of hemidesmosomes and skin blister formation was observed in *LAMA3* knock-out mice[23].

At the time of the initial visit to this family in 2016, we noticed the presence of reduced head circumference and severe ID. However, careful reevaluation of the patients after genetic testing also revealed the presence of blisters on the skin of hands and feet. This contrasts with previous findings where patients with *LAMA3* gene mutation presented Herlitz type junctional epidermolysis bullosa (JEB)[18, 19] and Laryngol-onycho-cutaneous syndrome[20,24]. The segregation analysis confirmed that the *LAMA3* gene variant (p. Arg1754Trp) is homozygous in affected individuals of our family, whereas unaffected individuals were either heterozygous or homozygous for wild type allele. This variant is reported in public databases in the heterozygous state (Table 1) but affected members of our family were homozygous and thus coincides with the autosomal recessive inheritance of phenotype in our family.

Microcephaly is a heterogeneous disorder that is characterized by small cortical size and reduction in the cortical convolution patterns[1, 2]. This reduction was associated with several genes malfunction that affects the specific pattern of cell division of neural progenitor cells and their migration to neocortical region[1]. At the beginning of neurogenesis, the neuro-epithelial cells give rise to radial glial cells (RGCs), these cells are self-renewing in nature and serve as a precursor for neural progenitors cells (NPCs) in the ventricular zone (VZ)[25]. With each division, one of the daughter cells

moves radially to the cortical plate, while one of the daughter cells remains in the VZ via its end feet attachment that spawns through the neocortical wall to the pial and ventricular surface[26-29]. These cellular attachments not only provide the anchorage to RGCs and maintain the precursor number but are also important for the migration of NPCs to the cortical plate[30, 31]. Nidogen, a laminin-binding cell adhesion protein is highly expressed in the RGCs and therefore may be important in the regulation of their division and migration. This led us to the presumption that laminin-5 (the protein encoded by the *LAMA3* gene) might play some role in the organization of RGCs and early neurogenesis[32]. Probably, laminin-5 plays role in embryonic cellular adhesion, division, and migration, and its expression in CNS prompts for additional studies to explore its role beyond recessive skin disorders.

CONCLUSION

This is the first report regarding the involvement of the *LAMA3* variant (p. Arg1754Trp) in autosomal recessive microcephaly and epidermolysis bullosa. None of the known genes for the microcephaly in recessive trait was observed in the same affected members of the family and only the *LAMA3* variant appears as a possible factor responsible for the phenotype of our family. This casual finding of the *LAMA3* variant in microcephaly patients is suggestive of its role in early cell division and migration of neural progenitor cells.

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AUTHOR CONTRIBUTION

Mughal ZU: Conceived of the study. Collected samples, conducted the research, organized, analyzed, and interpreted the data, and wrote the initial and final drafts of the article.
Zai JA: Collected, gathered, and managed data and also provide logistic support.
Ansar M: Conceived and supervised the study. Data interpretation. Critical revision, suggestions, and draft finalization.
All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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