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Research Article

WES Analysis Reveals a Nonsense Mutation in ASPM Gene Leading to Primary Microcephaly in a Saudi Arabian Family

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Abstract

Autosomal Recessive Primary Microcephaly (MCPH) is one of the most common hereditary neurological disorders in Saudi Arabia. Frequent consanguineous unions, due to isolated tribal set ups and large family sizes, is considered the primary reason for this high prevalence. In our study we ascertained a consanguineous family living in the South-Western region of the Kingdom. The patients were characterized by primary microcephaly, moderate intellectual disability and developmental delays. For genetic analysis we performed SNP array Based Comparative Genome Hybridization (aCGH) and analyzed the data for homozygosity mapping alongside Whole Exome Sequencing (WES). Three putative causal variants in regions of extended homozygosity on chromosome 1q31, chromosome 5p13.2 and chromosome 5p13.3 were identified: respectively, a nonsense variant in the *ASPM* gene (NM_018136; c.8903G>A; p.Trp2968*); a missense variant in *SPEF2* (NM_024867; c.1262G>A; p.Arg421His); and a missense variant in *PDZD2* (NM_178140; c.2153C>A; p.Thr718Asn) were found potential candidates for the disease phenotype in this family. Sanger sequencing determined that the variants in *SPEF2* and *PDZD2* did not co-segregate with the disease phenotype. However, the nonsense variant in *ASPM* does co-segregate with disease in this family. Our study concludes WES as a successful molecular diagnostic tool in this highly inbred population.

Introduction

Autosomal recessive primary microcephaly (MCPH) is a clinically and genetically heterogeneous neurodevelopmental disorder. The term "microcephaly" is used for a condition in which patients have a reduced head circumference and thus reduced brain size as compared to their age/sex matched controls. This small brain size is due primarily to the size reduction of the cerebral cortex; although architecturally the brain appears normal [1]. MCPH is usually associated with other symptoms such as intellectual disability, delayed speech and motor skills. Some affected children might have other features such as a narrow sloping forehead, seizures or short stature, as reviewed previously [2]. Before the advent of Next Generation Sequencing technologies (NGS), genetic diagnosis of autosomal recessive cases has primarily been performed or been identified via genome-wide homozygosity mapping followed by candidate gene sequencing. However, with the recent advancements, NGS technology has widely been used as a successful molecular diagnostic tool in these rare conditions including MCPH and others neurological disorders [3-5]. So far 19 genes have been identified for MCPH and related phenotypes and half of these cases have involved WES analysis for the causative variant identification [3,5]. We very recently discovered a new candidate gene for a novel syndromic form of primary microcephaly in a Saudi family using the combination of genome-wide homozygosity mapping with WES analysis [6].

In this study we ascertained a consanguineous family (Figure 1) which resided in South-Western region of Saudi Arabia segregating MCPH and took the same approach of homozygosity mapping combined with WES to provide a swift genetic diagnosis after having excluded microdeletions and micro duplications as a cause. This approach led to the identification of a mutation in *ASPM*.

Materials and methods

Ethical approval

Prior to the commencement of this study, an informed consent was signed by the legal guardians of the patients and unaffected participants. The study was approved by biomedical research and

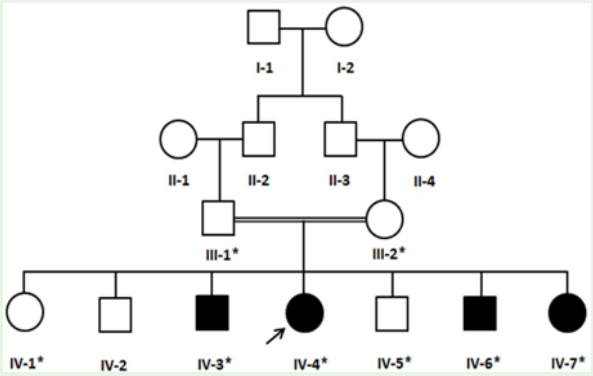

Figure 1: Pedigree drawing of a consanguineous family from Saudi Arabia showing autosomal recessive mode of inheritance. Arrow indicates the index patient. Asterisks indicate the samples which were available for the genetic analysis.

ethical committee (ref. # 24-14), King Abdulaziz University, Jeddah, Saudi Arabia.

Patients enrollment

The patient IV-4 was referred to the department of genetic medicine for genetic consultation by staff at the pediatrics clinic, King Abdulaziz University Hospital (KAAUH), Jeddah. Before referring to us the index patient IV-4 had been tested for targeted *ASPM* gene sequencing which according to the report was negative. The sample IV-5 was mentioned as an affected sibling with the clinical description of similar features as the other affected members in the family. Peripheral blood samples of the family were collected in EDTA tubes and were provided by the referring pediatrics consultants upon request. Genomic DNA of the available samples was extracted using standard methods through QIAamp blood DNA mini kit (QIAGEN GmbH, Germany).

Patients' history and clinical features

The family elders originated from Yemen and resided in the South Western region of Saudi Arabia. There were four affected out of seven siblings. The disease phenotype segregated in autosomal recessive mode of inheritance. All the affected individuals in this family had microcephaly with delayed speech, and mild to moderate intellectual disability. There were mild facial dysmorphic features with characteristic sloping forehead, sparse lateral eye brows, and slightly posteriorly rotated ears with a prominent anti helix and prominent antitragus, and short philtrum (Figure 2). The occipitofrontal circumference was 42 cm and the measurement of inner callus was 2.5 cm, palpebral fissure was 3 cm and outer callus was 10 cm. The brain Magnetic Resonance Imaging (MRI) of the index patient IV-4, revealed a mild form of lissencephaly.

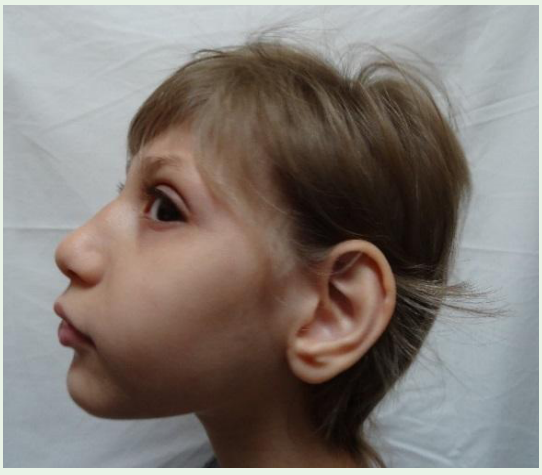

Figure 2: Clinical presentation of the affected child IV-4. The picture shows sparse lateral exe brows, sloping forehead, slightly posteriorly rotated ears with a prominent anti helix and prominent antitragus. It also shows the lightly pigmented hair.

Chromosomal analysis and homozygosity mapping

The Comparative Genome Hybridization (aCGH) analysis and genome wide homozygosity mapping were performed using Human CytoSNP12.2 chip, containing 300K SNPs throughout the human genome, together with the Illumina iScan platform (Illumina, San Diego, CA, USA). Copy Number Variation (CNV) analysis to detect chromosomal aberration, for example gross deletions/insertions, was performed by comparing the samples in the question with an in-house database assembled by Blue fuse software (Illumina, San Diego, CA, USA), as reported previously [7]. Regions of Homozygosity (ROH) were identified using the Loss of Heterozygosity (LOH) detection tool of the Genome Studio software (Illumina, San Diego, CA, USA).

Whole exome sequencing

Genomic DNA (2µg) from two siblings (IV-3 and IV-4) was subjected to whole exome paired-end sequencing analysis with 100× coverage by generating 51 Mb Sure Select V4 libraries (Agilent Technologies, USA). The sequence reads were run on a HiSeq2500 platform (Illumina, San Diego, CA, USA). Putative causal variants were identified according to the following criteria:

(a) Those variants with a minor allele frequency higher than 0.05 and Phred score less than 20 were excluded;

(b) Homozygous or compound heterozygous variants in exonic and splicing regions were included (based on the assumption of autosomal recessive inheritance);

(c) Variants not matching to the regions of homozygosity were excluded;

(d) Variants found in unrelated in-house exomes (n= 39) data were also filtered out. The shortlisted variants were further tested for likely pathogenicity using *in silico* prediction tools like MutationTaster2 [8] and Provean [9]. The selected variants were then genotyped using Sanger sequencing in all available family members to validate the WES data and to determine whether or not the variant in question co-segregated with disease status. A sum of 200 ethnically matched control chromosomes was tested to exclude the common polymorphisms of Saudi population.

Results and Discussion

SNP array genotyping

Genome-wide SNP microarray genotyping for one parent (mother III-2) and four siblings (IV-3, IV-4, IV-5 and IV-6) revealed four homozygous regions overlapping among the siblings on

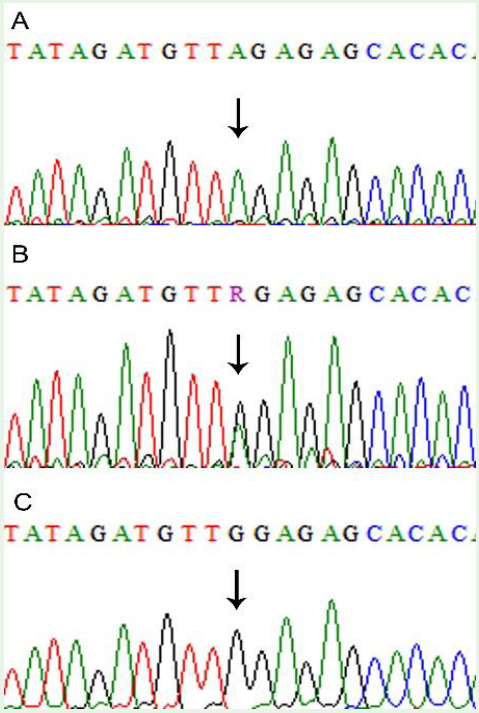

Figure 3: Sanger sequencing analysis of the *ASPM* mutation c.8903G>A in affected (A), unaffected parents or carriers (B) and healthy controls or wild type (C) individuals.

chromosomes 1q31.1-1q42.13, 1q23.1-1q24.3, 5p13.2-5p13.1 and 5q34-5q35.1 (Figures S1-S2). These regions contained 647 coding genes on chromosome 1 and 87 coding genes on chromosome 5 (Table1). Keeping in view the initial status of the siblings in the first referral the SNP genotyping results were unexpected as IV-5 was not matching to other siblings in homozygosity on chromosome 1, and similarly IV-3 was different from others on chromosome 5. Having no common homozygous region in all affected or having multiple overlapping regions was not surprising to us whilst using 300K Human CytoSNP12.2 genotyping chip in Illumina iScan platform, because we had observed similar familial cases in other families from Saudi Arabia [10,11]. Thus whole exome sequencing was suggested for at least two affected individuals (IV-3 and IV-4) who shared homozygous regions on the two chromosomes 1 and 5.

Causative variant identification

Candidate variants in Table 1 were filtered using the stated criteria. Sanger sequencing of these variants confirmed that the transversion mutation in ASPMgene at c. DNA position 8903 substituting guanine

Table 1: List of regions of homozygosity shared by the affected individuals in the family.

No	Chromosome	Physical position	Genes within the regions of homozygosity
1	1q31.1-1q42.13	1:193625278-226920773	647 genes including *ASPM*
2	1q23.1-1q24.3	1:161967287-178989464	
3	5p13.2-5p13.1	5:31675161-38399161	87genes including *PDZD2*and *SPEF2*
4	5q34-5q35.1	5:161221088-167543292	

Table 2: The position and likely pathological impact of homozygous variants in ASPM identified through WES.

Exon	c.DNA	Amino acid change	Mutation taster2
18	c. 8903G>A	p. Trp2968*	Disease causing
18	c.7939C>A	p.Leu2647Ile	Polymorphism
18	c.7684A>G	p.Ser2562Gly	Polymorphism
18	c.7480T>C	p.Tyr2494His	Polymorphism

with adenine (c.8903G>A) predicted as a premature termination codon at amino acid 2968 (p.Trp2968*) segregated correctly with the disease phenotype except sample IV-5 who were heterozygous for this variant (Figure 3). We then sequenced 200 ethnically matched control chromosomes and found that *ASPM* alteration (c.8903G>A) was not found outside the family. This mutation had been reported earlier from Middle East; however, the ethnicity of that single patient had not been disclosed [12].

The other variants in PDZD2 (c.2153C>A) and SPEF2 (c.1262G>A) found on chromosome 5 did not segregate with the disease phenotype (Table 3); therefore, they can be excluded from consideration as causal mutations in our patients.

ASPM function and prevalence

The *ASPM* gene includes 28 exons, with 62 kb of genomic sequence and the protein encodes 3,477 amino acids. *ASPM* encodes the abnormal spindle microtubule assembly protein which plays a very important role in normal mitotic spindle function in embryonic neuroblasts. In addition, the division of neural progenitor cells (early brain cells) is controlled by *ASPM* and is responsible for the overall size of the brain [13]. The Aspm gene in mouse is expressed in the primary sites of prenatal cerebral cortical neurogenesis [14]. Evolutionary studies have revealed that genes like *ASPM,* regulating brain size, have prominent role in brain development and can have contribution to brain evolution in primates and specifically in humans [15].

ASPM is a well-known gene in primary microcephaly and has been considered highly associated with MCPH [16]. There are 135 mutations identified so far in *ASPM* gene causing MCPH worldwide (TablesS1-S6). Most of these mutations are premature protein truncation with complete loss of *ASPM* function. In a large cohort study of 400 MCPH patients 12], the most common gene detected was *ASPM* accounting for 36% individuals of Middle Eastern and 42% individuals of South Asian descent. Most identified *ASPM* mutations showed no phenotype-genotype correlation [16,14]. In agreement with other studies, (Darvish, 2014) investigated 112 consanguineous Iranian families of MCPH children, *ASPM* was the most prevalent locus of the known MCPH loci. However, *ASPM* mutations were less prevalent compared to the Pakistani's population.

Table 3: Sanger sequencing for the variants inSPEF2 and PDZD2.

Variants from the exome data	Genotypes of the individuals screened through Sanger sequencing					
	III-1	IV-3	IV-5	IV-6	IV-7	Wild type
SPEF2	A/G	A/A	G/G	A/G	G/G	G/G
PDZD2	C/C	A/A	C/C	A/C	A/C	C/C

Sanger seuencing has been performed for 5 samples III-1(father), IV-3 (affected), IV-5 (unaffected), IV-6 (affected) and IV-7 (affected).

Conclusion

Genetic mutational analyses of *ASPM* and microcephaly have demonstrated that the majority of causal mutations are premature protein truncations, leading to complete loss of function of the normal protein (Supp. Tables S1-S3, S5). Previously most ASPM mutations were identified using linkage analysis method [16-20]. However, in our study a combination of homozygosity mapping and exome sequencing were used to detect the underlying causative variant. Our results identified a previously reported ASPM nonsense mutation which caused primary microcephaly due to a premature termination codon resulting in truncated or nonfunctional protein [21]. This stop codon alteration has been detected only in a single patient from Middle Eastern origin [12] but not specifically from Saudi and Yemeni populations. We believe that the strategy used in this study, i.e. the combination of homozygosity mapping and exome sequencing, is a powerful molecular diagnostic tool [5] and can be efficiently used in identifying causative variants in cases of microcephaly in highly inbred populations like Saudi Arabia.

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