

# A novel *WDR62* mutation causes primary microcephaly in a large consanguineous Saudi family

Muhammad Imran Naseer,<sup>a</sup> Mahmood Rasool,<sup>a</sup> Sameera Sogaty,<sup>b</sup> Rukhaa Adeel Chaudhary,<sup>c</sup> Haifa Mansour Mansour,<sup>c</sup> Adeel G. Chaudhary,<sup>a</sup> Adel M. Abuzenadah,<sup>a</sup> Mohammad H. Al-Qahtani<sup>a</sup>

From the <sup>a</sup>Center of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah, Saudi Arabia; <sup>b</sup>Department of Medical Genetics, King Fahad General Hospital, Jeddah, Saudi Arabia; <sup>c</sup>Faculty of Applied Medical Sciences, Medical Laboratory Technology, King Abdulaziz University, Jeddah, Saudi Arabia

Correspondence: Muhammad Imran Nasser · Center of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah 80216, Saudi Arabia · T: 96626401000-25486 F: 96626952521 · mimrannaseer@yahoo.com · ORCID: <http://orcid.org/0000-0002-9343-5825>

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**BACKGROUND:** Primary microcephaly (MCPH) is a rare developmental defect characterized by impaired cognitive functions, retarded neurodevelopment and reduced brain size. It is genetically heterogeneous and more than 17 genes so far have been identified that are associated with this disease.

**OBJECTIVE:** To study the genetic defect in a consanguineous Saudi family with primary microcephaly.

**DESIGN:** Cross-sectional clinical genetics study of a Saudi family.

**SETTING:** Medical genomics research center.

**PATIENTS AND METHODS:** Blood samples collected from six members of a family of healthy consanguineous parents were analyzed by whole exome sequencing to identify the underlying pathogenic mutations in two members of the family (23-year-old female and 7-year-old male) who presented with primary microcephaly, intellectual disability, delayed psychomotor development and walking difficulty, speech impediments and seizures.

**MAIN OUTCOME MEASURE(S):** Detection of mutation in the WD repeat domain 62 (*WDR62*) gene in a family segregating autosomal recessive primary microcephaly.

**RESULTS:** The exome variant analysis identified a novel missense mutation (c.3878C>A) in *WDR62* gene in exon 30 resulting in amino acid change from alanine to aspartate (p.Ala1293Asp). Further validation in the affected patients and healthy members of family and 100 unrelated healthy persons as controls confirmed it to be pathogenic.

**CONCLUSIONS:** Functional impairment of the *WDR62* gene can lead to severe neurodevelopmental defects, brain malformations and reduced head size. A missense mutation of exon 30 changed alanine to aspartate in the *WDR62* protein leading to the typical MCPH phenotype.

**LIMITATIONS:** Mutation was identified in a single family.

**M**icrocephaly (MCPH) is an abnormally small head size. There are two sub categories: primary microcephaly is present at birth and is a static developmental anomaly; secondary microcephaly develops later in life (postnatally) and is a progressive neurodegenerative condition. Primary microcephaly is a congenital condition associated with incomplete brain development, where a baby's head is smaller than expected when compared to babies of

the same sex and age. It serves as an important neurological indication or warning sign, but no uniformity exists in its definition. It is usually defined as a head circumference (HC) more than four standard deviations (SD) below the mean for age and sex.<sup>1</sup>

Primary microcephaly (MCPH) is very rare; the rate is estimated to be 1 in 30000 in Japan<sup>2</sup> and 1 in 250000 in Holland.<sup>3</sup> This rate increases to 1 in 10000 in regions where interfamily and cousin marriages are

common.<sup>4</sup> The birth incidence varies from 1.3 to 150 per 100 000 persons, depending on various factors, mainly population type and consanguinity.<sup>5</sup>

So far, 17 genes have been identified that underlie autosomal recessive primary microcephaly. These include microcephalin at MCPH1,<sup>6</sup> *WDR62* at MCPH2,<sup>7-9</sup> *CDK5RAP2* at MCPH3,<sup>10</sup> *CASC5* at MCPH4,<sup>11</sup> *ASPM* at MCPH5,<sup>12</sup> *CENPJ* at MCPH6,<sup>12</sup> *STIL* at MCPH7,<sup>13</sup> *CEP135* at MCPH8,<sup>14</sup> *CEP152* at MCPH9,<sup>11,15</sup> *ZNF335* at MCPH10,<sup>16</sup> *PHC1* at MCPH11,<sup>17</sup> *CDK6* at MCPH12,<sup>18</sup> *CENPR* at MCPH13,<sup>19</sup> *SASS6* at MCPH14,<sup>20</sup> *MFSD2A* at MCPH15,<sup>21</sup> *ANKLE2* at MCPH16,<sup>22</sup> *CIT* at MCPH17.<sup>23-25</sup> Furthermore, recently identified syndromic microcephaly *AGMO*, *RTTN* and *PGAP2* genes have been reported in the Saudi population.<sup>26-28</sup>

However, the majority of mutations have been identified in two genes: *ASPM*, accounting for more than half of all mutations and *WDR62*, which accounts for around 10% of all cases.<sup>29</sup> All of the *WDR62* mutation cases presented up to now have shown the presence of mental retardation and also the presence of prominent microcephaly on physical examination, while some of the patients also suffered from seizures. The examination of the brain of the patients under high field strength (3 Tesla) magnetic resonance imaging (MRI) have identified hallmarks of a wide range of severe cortical malformations.<sup>8</sup>

In our study, we have ascertained the presence of a novel mutation in a consanguineous family from Saudi origin. There were two affected siblings born to a consanguineous union in this family. Whole exome sequencing was performed to identify the underlying genetic cause because of the potential of this technology in the molecular diagnostics of similar genetic disorders.<sup>30</sup> We identified a novel mutation in the *WDR62* gene in these Saudi patients.

## PATIENTS AND METHODS

### Sample collections

The pedigree (family chart) of the family was drawn by obtaining information from elders of the family (**Figure 1**). Pedigree construction suggested an autosomal recessive pattern of inheritance (**Figure 1**). Before the study was initiated, written informed consent was taken from all participants of the study. The study was also approved from ethical committee of the Center of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah (013-CEGMR-2-ETH). The blood samples were collected from six members of the family (two affected and four normal individu-

als) and one hundred unrelated healthy people of Saudi origin as controls. Both the affected individuals underwent medical examination at King Abdulaziz University Hospital, Jeddah. We ruled out possible environmental factors and infections such as rubella, toxoplasma, cytomegalovirus, and Zika virus that may lead to microcephaly.

### Patient 1

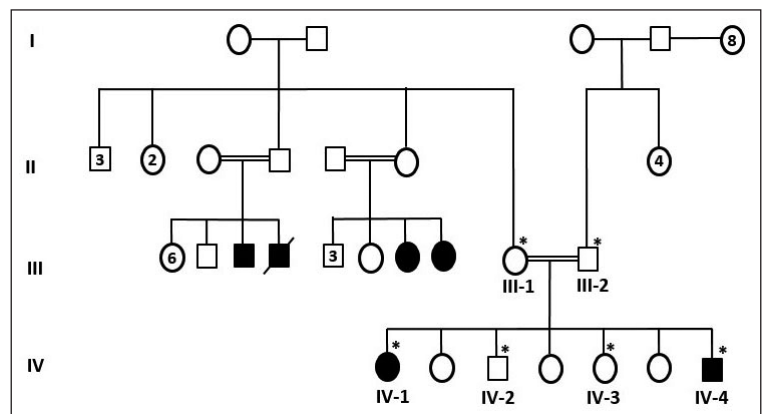
Proband (IV-1) was a 23-year-old female who presented with MCPH and suffered from mental retardation. She was the first child of consanguineous healthy parents (**Figure 1**). She had a normal weight and height, but could not walk properly, had speech problems, had brain atrophy and reported seizures. Her head circumference was less than 5 standard deviations below the mean.

### Patient 2

Proband (IV-4) was a 7-year-old male who presented with MCPH and suffered from mental retardation. This individual was born after five normal offspring as shown in the pedigree (**Figure 1**). He was not able to move due to muscular dystrophy, could not speak properly, and had a history of seizures. The head circumference was less than 5 standard deviations below the mean.

### Whole exome sequencing

To identify the underlying pathogenic mutation behind this disease phenotype we planned whole exome sequencing (HiSeq 2500 System, Illumina, San Diego, CA, United States). Extraction of genomic DNA (gDNA) was performed using standard procedures.



**Figure 1.** A pedigree of a consanguineous family from Saudi Arabia showing the disease phenotype segregating in an autosomal recessive manner. The samples available for genetic testing are marked with asterisks.

Briefly, the blood sample was used for direct gDNA isolation using the QIAamp DNA Blood Mini Kit, Cat. Nr. 51106 (Qiagen, Hilden, Germany) following manufacturer's instructions and modified where necessary. The gDNA was analyzed by the Bioanalyzer system (Agilent Technologies, Santa Clara, CA, United States) and quantified by a NanoDrop Spectrophotometer and the genomic DNA was stored under appropriate conditions for future analysis.

The samples were prepared according to an Agilent SureSelect Target Enrichment Kit preparation guide (Capture kit, SureSelect\_v6) by using genomic DNA directly. The genomic DNA libraries were sequenced using the Illumina HiSeq 2000/2500 sequencer. The resulting VCF (variant call format) file contained 89064 variants. These variants were filtered based on quality, frequency, genomic position, protein effect, pathogenicity and previous associations with the phenotype.

### Sanger sequencing

To confirm the mutation in patients and family, we did Sanger sequencing (ABI 3700). To confirm the mutation as pathogenic, we also sequenced this DNA variant in 100 unrelated control people.

## RESULTS

Candidate variants were first searched in a broad panel of genes which had been previously associated with microcephaly, intellectual disability, and one of the reported features (in human and model organisms). The exome variant analysis yielded a plausible candidate variant in the *WDR62* gene where cytosine

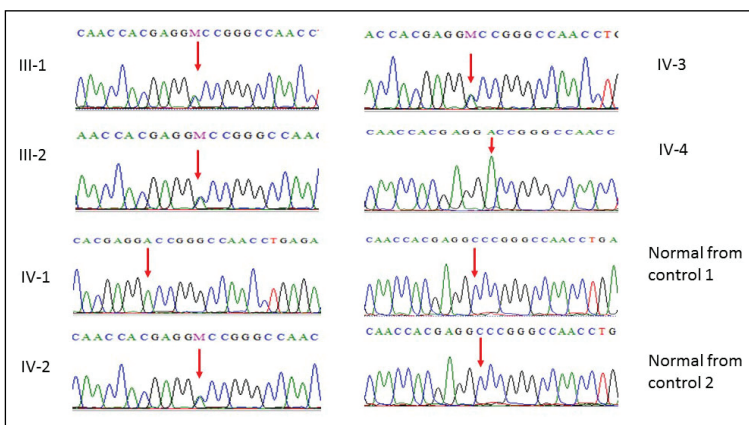
(C) at position 3878 is replaced by adenine (A), resulting in conversion of amino acid alanine at position 1293 to aspartate, thus showing a novel missense mutation 3878C>A, at exon 30 in affected members of the family as shown in **Figure 2**. Furthermore, in the Greater Middle East variome, the minor allele frequency was 0.0005 and there was single heterozygous and no homozygous individuals found in the database. Moreover, PolyPhen, MutationTaster and SIFT predicted this disease-causing mutation. This mutation was absent in the Human Gene Mutation database (HGMD, [www.hgmd.cf.ac.uk/](http://www.hgmd.cf.ac.uk/)) and MIM. 1000 genome (<http://www.internationalgenome.org/>) and Exome Aggregation Consortium (<http://exac.broadinstitute.org/>) database.

Other mutations in this gene are known to cause primary autosomal recessive microcephaly type 2, with or without cortical malformations (MCPH2). This disorder shows phenotypic overlap with the symptoms reported for this individual. Hence, this variant was considered as a plausible candidate, but needed further investigation to validate its clinical significance.

To check the other mutations elsewhere in the whole exomes, we also broadened the analysis to all genes applying all inheritance modes. However, no additional potential candidate variant that might be of relevance for the reported phenotype could be identified. To confirm this novel mutation in *WDR62* gene, further validation was carried out in all available affected patients and healthy members of family and 100 unrelated healthy persons as controls. This mutation was not detected in any healthy individual, which confirmed it to be pathogenic. The parents of the affected members were heterozygous, which also confirms the autosomal recessive mode of inheritance.

## DISCUSSION

To date about 25 mutations have been identified in *WDR62* gene (**Table 1**). In our family, a rare, homozygous missense variant was detected in the *WDR62* gene in patients in a homozygous state. This gene encodes a protein which is required for proper neurogenesis and cerebral cortical development.<sup>31</sup> It is proposed to play a role in neuronal proliferation and migration.<sup>7,8</sup> The expression of *WDR62* was found to be widespread in the developing mouse brain, with highest expression in the forebrain.<sup>7</sup> Mechanistically, *WDR62* associates and genetically interacts with Aurora A to regulate spindle formation, mitotic progression and brain size. It is also reported that *WDR62*



**Figure 2.** Sanger sequence analysis: mother III-1, father III-2, IV-2, IV-3 are normal parents showing C and A in heterozygous state, while IV-1 and IV-4 are affected children showing only homozygous A in exon 30 of *WDR62* gene.

**Table 1.** Mutation spectrum of *WDR62* gene mutations.

S. No	Nucleotide variation	Amino acid variation	Location	Reference
1	c.28G>T	p.Ala10Ser	Exon 1	41
2	c.189G>T	p.Glu63Asp	Exon 2	41
3	c.193 G>A	p.Val65Met	Exon 2	7, 9
4	c.332G>C	p.Arg111Thr	Exon 3	19, 29
5	c.363delT	p.Asp112MetfsX5	Exon 4	7
6	c.535_536insA	p.Met179fsX21	Exon 5	33
7	c.671 G>C	p.Trp224Ser	Exon 6	8
8	c.900C>A	p.Cys300X	Exon 8	33
9	c.1043+1 G>A	p.Ser348RfsX63	Intron 8	7
10	c.1143delA	p.H381PfsX48	Exon 9	42
11	c.1194G>A	p.Trp398	Exon 9	18, 29
12	c.1313 G>A	p.Arg438His	Exon 10	9
13	c.1408C>T	p.Gln470X	Exon 11	8
14	c.1531 G>A	p.Asp511Asn	Exon 11	9
15	c.1576 G>T	p.Glu526X	Exon 12	8
16	c.1576 G>A	p.Glu526Lys	Exon 12	8
17	c.1942 C>T	p.Gln648X	Exon 15	43
18	c.2867+4_c2867+7delGGTG	p.Ser956CysfsX38	Intron 23	7
19	c.3232 G>A	p.Ala1078Thr	Exon 27	9
20	c.3361delG	p.Ala1121Glnfs*6	Exon 28	18, 29
21	c.3503G>A	p.Trp1168*	Exon 29	18, 29
22	c.3839_3855delGCCAAGAGCCTGCCCTG	p.Gly1280AlafsX21	Exon 30	7, 8
23	c.3878C>A	p.Ala1293Asp	Exon 30	This study
24	c.3936dupC/3936_3937incC	p.Val1314ArgfsX18/ Val1314GlyfsX17	Exon 30	7, 9
25	c.4205delTGCC	p.Val1402GlyfsX12	Exon 31	8
26	c.4241dupT	p.Leu1414LeufsX41	Exon 31	9

interacts with Aurora A to control mitotic progression, and loss of these interactions leads to mitotic delay and cell death of neural progenitor cells (NPCs) which could be a potential cause of human microcephaly.<sup>32</sup> Mutations in this gene have been associated with microcephaly 2, primary, autosomal recessive, with or without cortical malformations (MCPH2).<sup>7-9,33-36</sup> This is a disease characterized by microcephaly associated with other manifestations and shows wide phenotypic variability.<sup>37</sup> Associated features include moderate to severe mental retardation, and various types of cortical malformations in most patients. Cortical

malformations may include pachygyria with cortical thickening, microgyria, lissencephaly, hypoplasia of the corpus callosum, and schizencephaly. All affected individuals have delayed psychomotor development. Some patients have seizures.

The variant detected here is a substitution that affects a highly conserved alanine residue that is the last amino acid of the last WD domain (WD stands for tryptophan-aspartic acid dipeptide). The underlying common function of all WD-repeat proteins is coordinating multi-protein complex assemblies, where the repeating units serve as a rigid scaffold

**Table 2.** In silico tools used for prediction of pathogenicity of missense variants.

S. No	Online tools	Pathogenicity score
1	Ensembl ( <a href="http://www.ensembl.org">http://www.ensembl.org</a> , ENST00000401500)	0.0
2	SIFT ( <a href="http://sift.jcvi.org/">http://sift.jcvi.org/</a> )	1.0
3	1000 Genomes ( <a href="http://www.internationalgenome.org/">http://www.internationalgenome.org/</a> )	0.0
4	Exome Aggregation Consortium ( <a href="http://exac.broadinstitute.org/">http://exac.broadinstitute.org/</a> )	0.0
5	Polyphen -2 ( <a href="http://genetics.bwh.harvard.edu/pph2/">http://genetics.bwh.harvard.edu/pph2/</a> )	0.99
6	MutationTaster ( <a href="http://www.mutationtaster.org/">http://www.mutationtaster.org/</a> )	1.1
7	MutationAssessor	0.93
8	PhyloP ( <a href="https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4702902/">https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4702902/</a> )	0.76
9	GERP++ ( <a href="http://mendel.stanford.edu/SidowLab/downloads/gerp/">http://mendel.stanford.edu/SidowLab/downloads/gerp/</a> )	0.97

for protein interactions. This mutation was not previously reported. Previously reported pathogenic mutations include missense (e.g. E526K; W224S; R438H) and truncating mutations (e.g. Val1402GlyfsTer12; Q470X; Gly1280AlafsTer21; 2083delA; 2472\_2473delAG; c.390G>A; c.2527dupG; p.R438H; p.D955Afs\*112).<sup>8,9,34,35,38-40</sup> The frameshift mutations reported by Murdock et al were reported to cause nonsense-mediated mRNA decay and loss of function.<sup>34</sup> However, the effect of the missense variant detected here remains to be elucidated. Interestingly, this variant is predicted to be deleterious by the majority of in silico prediction tools. This variant is absent from population databases such as 1000 Genomes Project and the Exome Aggregation Consortium. The variant was tested for likely pathogenic effect by in silico tools like MutationTaster,<sup>44</sup> SIFT and Polyphen, and it was predicted as "disease causing" with high pathogenicity scores (**Table 2**). Further functional analysis is needed to determine the functional effect of this variant and its possible contribution to the reported phenotype.

**Conflict of interest**

The authors declare no conflict of interest.

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