Clinical Vignette

Exome Sequencing Reveals a Novel Homozygous Variant in *WDR62* Gene in a Family with Primary Microcephaly

Ikrormi Rungsung[1], Mahesh Kamate[2], Ashwin Dalal[1]

[1]*Diagnostics Division, Centre for DNA Fingerprinting and Diagnostics, Hyderabad, India*

[2]*Department of Pediatric Neurology, Jawaharlal Nehru Medical College, KLE University, Belgaum, Karnataka, India*

Correspondence to: Dr Ashwin Dalal *Email:* ashwindalal@gmail.com

Abstract

Autosomal recessive primary microcephaly 2 (MCPH2) is a neurodevelopmental disease that causes reduction in brain size. Homozygous or compound heterozygous mutations in the *WDR62* gene, located at the chr19q13.12 locus are reported to result in MCPH2. The most common features are reduced skull circumference and intellectual disability with or without cortical malformations. We describe a genetic variant in two siblings, a 4-year-old boy and a 15-month-old girl, with congenital microcephaly, global developmental delay, intellectual disability and hyperactivity. Exome sequencing was performed on the genomic DNA and analyses revealed a novel frameshift deletion, NM_001083961.2; c.669delC; p. Phe223fs in exon 6 of the *WDR62* gene. The variant c.669delC causes a frameshift at p. Phe223fs position of the WD40-repeat 62 protein (WDR62) protein and is classified as a 'pathogenic' variant according to the American College of Medical Genetics/ Association for Molecular Pathology (ACMG/AMP) classification. The unaffected parents were found to be heterozygous for this mutation. Our findings expand the mutation spectrum of *WDR62* gene-related phenotype.

Introduction

The worldwide incidence of microcephaly varies from 1.3 to 150 per 100,000 populations (Tolmie et al., 1987). Microcephaly has been reported more commonly in Asians and Arabs due to consanguineous unions (Hussain & Bittles, 1998; Thornton & Woods, 2009; Woods et al., 2005). Autosomal recessive primary microcephaly (MCPH) is characterized by a small head circumference ranging from 2 standard deviations (SD) to 11 SD below the mean for age and sex-matched individuals. The affected patients show delayed psychomotor development and mild to severe intellectual disability, which is often accompanied by other brain malformations. The Online Mendelian Inheritance in Man (OMIM) has reported twenty-seven loci or genes for primary microcephaly. It has been reported that most of the MCPH-associated gene products are centrosomal proteins and play diverse roles during neurogenic mitosis (Cox et al., 2006). Here we report a novel homozygous frameshift variant in *WDR62* gene in two siblings born to consanguineous parents, identified through exome sequencing. *WDR62* gene is expressed in the neuroepithelium of apical precursors during mitosis (Nicholas et al., 2010).

Patient details

The proband is a 4-year-old boy, born at term gestation, with a birth weight of 3.5 kg. He was noted to have small head size at birth. There is second degree consanguinity in the parents (Figure 3A). Antenatal and perinatal periods were uneventful. There was global developmental delay with sitting attained at 10 months and walking at 2 years. He spoke his first word at 3 years and had drooling of saliva from 2 years of age. He was first seen at 15 months of age and was on follow-up thereafter. At the time of initial examination, the head circumference was 37.0 cm (z-score –7.5), height was 73 cm (z-score –3) and weight was 7.5 kg (z-score –3.3). Head circumference at 3.5 yrs was 39.0 cm (z score –6.7), height was 87 cm (z-score –3.3) and weight was 8.0 kg

(z-score –4.8). Currently patient has hyperactivity and aggressiveness. He has left hand preference. There was no history of seizures. MRI brain was normal with simplified gyral pattern. There was no ventriculomegaly, lissencephaly, polymicrogyria or cerebellar dysplasia. The thickness of the cortex was also normal. The second sibling, a female child, who is now 15 months of age, weighed 2.0 kg at birth and was also noted to have a small-sized head. She also has global developmental delay (attained head control at 4 months; but cannot sit or stand and has not attained language milestones), and her head circumference was 36.5 cm (z score –6.6), height was 63 cm (z-score –3.3) and weight was 5.7 kg (z-score –3.8) at 10 months of age. She had drooling of saliva with normal tone and no seizures. Both siblings showed mild dysmorphic features in the form of triangular facies, broad nasal root, bulbous tip of nose, smooth philtrum, thin upper lip and thick lower lip, and smooth philtrum (Figure 1).

Figure 1 Photographs of proband and sibling showing microcephaly.

Genomic DNA isolation

We collected 2 milliliter of peripheral blood sample in EDTA vacutainer tube (BD-Plymouth, PL6 7BP, UK) from the proband, his sibling and both parents, after obtaining informed consent. Total genomic DNA was isolated from blood using the HiGEnoMB DNA purification kit (HiMedia Laboratories, LLC).

Whole–exome sequencing

Exome sequencing was performed on genomic DNA. The SureSelect Clinical Research Exome V2 kit (Agilent SureSelect technology) was used to capture and enrich regions from exons along with 75,000 splice sites of non-coding exons, more than 12,000 deep intronic sites and over 800 promoter regions. The captured and enriched library was amplified and sequenced on the Illumina sequencer, for 100X coverage. The reads were assessed for quality control using the FastQC and mapped to the human reference genome 19 (hg19/GRCh37) using the BWA MEM program. The variant calling was done using GATK haplotypecaller and the VCF file was annotated against the genomic variation population databases and bioinformatics prediction tools. The population databases used were 1000 Genomes, gnomAD, Exome Variant Server, GenomeAsia, in house databases and the bioinformatics prediction tools used were MutationTaster and Combined Annotation Depletion Dependent (CADD).

Sanger sequencing

Specific primers F1 and R1 were designed for the *WDR62* gene mutation and amplified. PCR products were visualized in 2% agarose gel electrophoresis and then sequenced on ABI Prism A3730-automated sequencer (PE Applied Biosystems, Thermo Fisher Scientific, Waltham MA, USA). The Sanger sequence chromatograms were visualized with FinchTV (Geospiza, Inc. Seattle, WA, USA) for the presence or absence of the mutation.

Results

Exome sequencing was performed on the proband which revealed 131,974 total variants. To identify the causative variant, the polymorphic variants [with minor allele frequency (MAF) ≥ 0.01] present in the 1000 Genomes, ExAC, EVS, gnomAD, GME, cg69 and in-house exome databases, were excluded. Further, we looked for variants in the exonic regions and splice sites. This led to 22 non-synonymous variants, 2 stop-gain variants, 2 frameshift deletions and one frameshift insertion variant in homozygous state (Figure 2). A novel frameshift deletion variant (NM_001083961.2; c.669delC) in the *WDR62* (OMIM ID #604317 (`https://omim.org/`) gene was chosen as the candidate variant, because the reported *WDR62*-associated phenotype matched the proband's clinical features. The variant was submitted to ClinVar with the accession number VCV000818086.1. It is classified as 'pathogenic'

as per the ACMG/AMP guidelines (Figure 3B). *WDR62* gene mutations are known to cause autosomal recessive primary microcephaly 2, with or without cortical malformations (MCPH2). *In silico* prediction tools showed the variant to be disease-causing. Sanger sequencing confirmed the homozygous single base pair deletion in exon 6 at NM_001083961.2; c.669delC mutation in *WDR62* gene in both the proband and the sibling, and the same was found to be present in heterozygous form in both the unaffected consanguineous parents (Figure 3C).

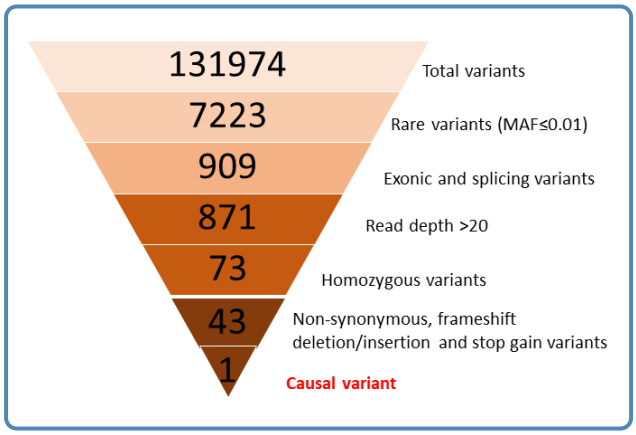

Figure 2 Filtering strategy of exome sequencing data.

Discussion

Microcephaly-2 with or without cortical malformations is inherited in an autosomal recessive fashion and shows significant phenotypic variability. Patients with pathogenic variants in *WDR62* have head circumferences ranging from low-normal to severe (–9.8 SD) microcephaly, and most patients reveal various types of cortical malformations in brain MRI. All patients have delayed psychomotor development but seizures are variable.

We have presented genetic evidence of a novel frameshift deletion in *WDR62* gene linking to autosomal recessive primary microcephaly 2 (MCPH2). Our data revealed that this frameshift deletion co-segregated with the disease phenotype, since it was present in the affected sibling in the homozygous state and in the heterozygous state in both parents. In humans, *WDR62* gene encodes for WD40-repeat protein 62 (WDR62) protein, which has 1518 amino acids and contains tryptophan-aspartic acid (WD) dipeptide repeats. Studies have shown that WDR62 protein binds with the centrosomal protein CEP170 and it stabilizes the mitotic spindle during metaphase. It is also known that the WDR62 protein plays a role in neurogenesis via the c-Jun N-terminal kinase (JNK) signaling pathway (Bhat et al., 2011).

There are reports of consanguineous families with microcephaly-2 with cortical malformations, including polymicrogyria, schizencephaly, and subcortical heterotopia. Another study from India reported on 2 different homozygous truncating *WDR62* mutations in unrelated consanguineous families with MCPH2 with cortical malformations (Bhat et al., 2011). Our patient did not have any brain malformations other than simplified gyral pattern.

The *WDR62* gene has 32 exons and different types of mutation like missense, nonsense, splice site and indels have been reported across the exonic regions (Figure 3D). Bilguvar et al have identified homozygosity for a 4-basepair deletion (TGCC) in exon 31 of the *WDR62* gene at codon 1402, G-to-A substitution in exon 12 at codon 526, G-to-C transversion in exon 6 at codon 224, C-to-T transition in exon 11 at codon 470, and a 17-bp deletion in exon 30 at codon 1280 (Bilgüvar et al., 2017). Homozygous 1313G-A transition in exon 10 and duplication 4241dupT in exon 31 of the *WDR62* gene were also reported by another group (Roberts et al., 1999). Yu et al have also reported homozygous 1531G-A transition in exon 11, 1-bp insertion (3936insC) in exon 30, 1-bp deletion (363delT) in exon 4 of the *WDR62* gene and a 193G-A transition in exon 2 of the *WDR62* gene (Yu et al., 2010). In addition, a 1-bp deletion (2083delA) in exon 17 and a 2-bp deletion at 2472_2473delAG in exon 23 of *WDR62* gene have also been reported (Nicholas et al., 2010).

Our results assert that NM_001083961.2; c.669delC variant in *WDR62* gene explains the clinical features of microcephaly, global developmental delay, intellectual disability and hyperactivity observed in the present sibship and expands the genotypic spectrum of variants in the *WDR62* gene.

Web resources

- OMIM, `https://www.omim.org/`
- MutationTaster, `http://www.mutationtaster.org/`

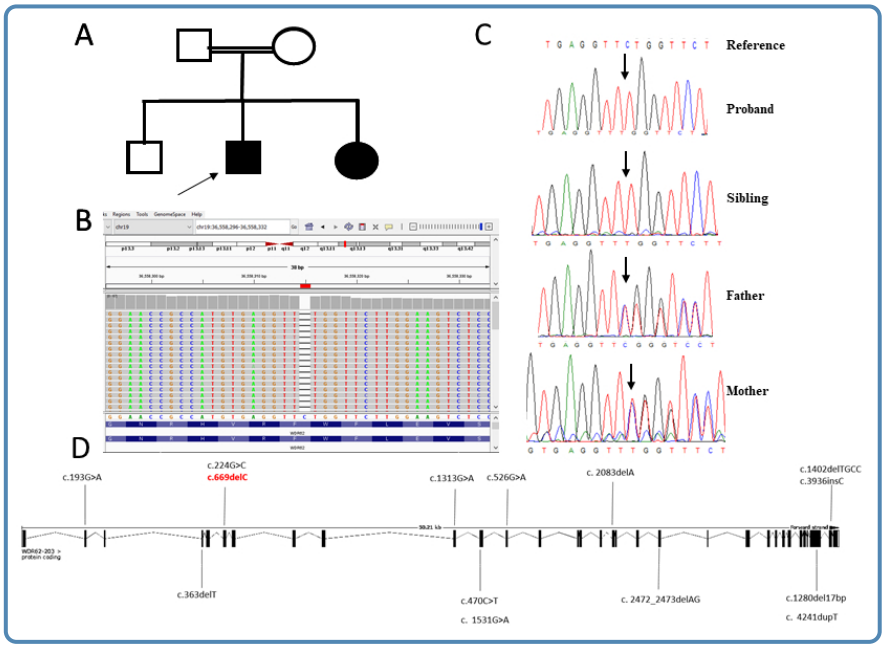

Figure 3 A. Pedigree of the family. B. Variant visualization using Integrated genome viewer for c.669delC variant in exon 6 of the *WDR62* gene. C. Segregation analysis using targeted Sanger sequencing in the patient's family. D. Schematic representation of all *WDR62* exons (adapted from the Ensembl browser) showing exon-wise known mutations with our novel variant in red (NM_001083961.2; c.669delC) for transcript ID ENST00000401500.7.

- CADD, http://cadd.gs.washington.edu/score
- 1000 Genomes Project, http://phase3browser.1000genomes.org/index.html
- gnomAD, http://gnomad.broadinstitute.org/
- GenomeAsia https://genomeasia100k.org/
- Exome Variant Server https://evs.gs.washington.edu/EVS/

References

1. Bhat V, et al. Mutations in WDR62, encoding a centrosomal and nuclear protein, in Indian primary microcephaly families with cortical malformations. Clin Genet. 2011; 80: 532–540.
2. Bilgüvar K, et al. Whole exome sequencing identifies recessive WDR62 mutations in severe brain malformations Nature. 2010; 467:207–210.
3. Cox J, et al. What primary microcephaly can tell us about brain growth. Trends Mol Med. 2006; 12: 358–366.
4. Hussain R, et al. The prevalence and demographic characteristics of consanguineous marriages in Pakistan. J Biosoc Sci. 1998; 30: 261–275.
5. Nicholas AK, et al. WDR62 is associated with the spindle pole and is mutated in human microcephaly. Nat Genet. 2010; 42: 1010–1014.
6. Roberts E, et al. The second locus for autosomal recessive primary microcephaly (MCPH2) maps to chromosome 19q13.1-13.2. Eur J Hum Genet. 1999; 7: 815–820.
7. Thornton GK, et al. Primary microcephaly: do all roads lead to Rome? Trends Genet. 2009; 25: 501–510.
8. Tolmie JL, et al. Microcephaly: Genetic counselling and antenatal diagnosis after the birth of an affected child. Am J Med Genet. 1987; 27: 583–594.
9. Woods CG, et al. Autosomal recessive primary microcephaly (MCPH): A review of clinical, molecular, and evolutionary findings. Am J Med Genet. 2005; 76: 717–728.
10. Yu TW, et al. Mutations in WDR62, encoding a centrosome-associated protein, cause microcephaly with simplified gyri and abnormal cortical architecture. Nat Genet. 2010; 42: 1015–1020.