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Whole-Exome Sequencing Identifies Compound Heterozygous Mutations in *WDR62* in Siblings With Recurrent Polymicrogyria

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Abstract

Polymicrogyria is a disorder of neuronal development resulting in structurally abnormal cerebral hemispheres characterized by over-folding and abnormal lamination of the cerebral cortex. Polymicrogyria is frequently associated with severe neurologic deficits including intellectual disability, motor problems, and epilepsy. There are acquired and genetic causes of polymicrogyria, but most patients with a presumed genetic etiology lack a specific diagnosis. Here we report using whole-exome sequencing to identify compound heterozygous mutations in the *WD repeat domain 62* (*WDR62*) gene as the cause of recurrent polymicrogyria in a sibling pair. Sanger sequencing confirmed that the siblings both inherited 1-bp (maternal allele) and 2-bp (paternal allele) frameshift deletions, which predict premature truncation of WDR62, a protein that has a role in early cortical development. The probands are from a non-consanguineous family of Northern European descent, suggesting that autosomal recessive PMG due to compound heterozygous mutation of *WDR62* might be a relatively common cause of PMG in the population. Further studies to identify mutation frequency in the population are needed.

Keywords

malformations of cortical development; high-throughput nucleotide sequencing; genetic testing; epilepsy; intellectual disability

INTRODUCTION

Polymicrogyria (PMG) is a CNS malformation characterized pathologically by numerous abnormal small gyri and shallow sulci and abnormal lamination of the cerebral cortex possibly due to impaired neuronal migration or proliferation during cortical development [Leventer et al., 2010]. Clinically, PMG is diagnosed when cranial magnetic resonance imaging (MRI) identifies an irregular appearing cortical surface, a thickened or overfolded cortex, and irregularity at the grey-white matter boundary [Leventer et al., 2010]. Polymicrogyria is a heterogeneous malformation that can be focal or diffuse, unilateral or

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bilateral, isolated or occurring with other cortical abnormalities [Jansen and Andermann, 2005]. Clinical manifestations are also variable but frequently include cognitive impairment, motor deficits, and epilepsy. Known causes of PMG include intrauterine insults such as infection or ischemia as well as genetic causes which include recurrent gene deletion syndromes or genetically linked loci in which the specific contributing gene has not been identified, including regions on chromosomes 1p36.3, 2p16.1–p23.1, 4q21.21–q22.1, 6q16–q22, 6q26–q27, 21q2, 22q11.2, and Xq28 [Villard et al., 2002; Robin et al., 2006; Dobyns et al., 2008; Ben Cheikh et al., 2009]. Specific gene mutations in *RAB3GAP1/2, TBR2, KIAA1279, PAX6, TUBB2B, SRPX2*, and *GPR56* have also been associated with PMG [Roll et al., 2006; Dobyns et al., 2008; Jaglin et al., 2009; Bahi-Buisson et al., 2010; Borck et al., 2011]. Recent reports have also implicated homozygous mutations in the *WDR62* gene in diverse brain malformations which often include polymicrogyria [Bilguvar et al., 2010; Nicholas et al., 2010; Yu et al., 2010]. Unlike the majority of cases of PMG that appear to be spontaneous, these genetic alterations were identified in consanguineous kindreds.

We recently demonstrated the use of high-throughput sequencing to identify a novel causative gene in a family with an autosomal recessive form of Charcot–Marie–Tooth disease [Lupski et al., 2010]. We also showed the utility of DNA capture-sequencing of the human exome in rare variant discovery [Bainbridge et al., 2010]. Here we describe the use of high-throughput whole-exome sequencing to identify compound heterozygous mutations in *WDR62* in two brothers from a non-consanguineous family with PMG.

METHODS

Clinical Ascertainment

Informed consent for participation, sample collection, and medical records review was obtained using protocols approved by the Institutional Review Board for Baylor College of Medicine (BCM) and affiliated hospitals. Clinical histories and physical examinations were obtained by one of the authors (board certified pediatric neurologist). DNA was extracted from peripheral blood using the Puregene DNA isolation kit (Gentra System, Minneapolis, MN).

Clinical Case Reports

Patient II-1 was born to a 37-year-old $G_1P_{0–1}$ mother at term via induced vaginal delivery. The pregnancy was complicated by gestational diabetes. Microcephaly was diagnosed in utero at 30 weeks gestation, and he was apparently microcephalic at birth (birth measurements were not available). Cranial MRI on day 13 of life demonstrated bilateral parietal polymicrogyria and an otherwise immature cortical gyral pattern (Fig. 1A–C). Developmentally, he was globally delayed. He developed infantile spasms at age four months, and he continues to suffer with medically intractable symptomatic generalized epilepsy. At age 8.8 years, head circumference was 46.5 cm (<5th centile), weight was 19.6 kg (<5th centile), and height was 109.2 cm (<5th centile). At his current age of 9 years, he also has intellectual disabilities and spastic quadriparesis.

Patient II-2 was born to the then 39-year-old $G_2P_{1–2}$ mother at term via planned caesarean. There were no complications during the pregnancy. Birth measurements were not available. Cranial MRI at age 4 years demonstrated an asymmetrically small left cerebral hemisphere with extensive polymicrogyria and a focus of gray matter heterotopia in the right parietal region (Fig. 1D–F); growth parameters at this age included head circumference 45 cm (<5th centile) and weight 13.7 kg (~5th centile). At his current age of 7 years, he has delayed social development and a mild right hemiparesis; but he is ambulatory, uses age-appropriate

language, is cognitively on target with his peers, and he has not developed epilepsy. Refer to Figure 1G for the pedigree.

Array comparative genomic hybridization using the BCM Medical Genetics Laboratories 180k oligonucleotide array V8.1 demonstrated normal genomic copy-number in both brothers.

Exome Capture and Sequencing, Read Mapping, and Variant Annotation

A whole exome-enriched library was prepared from genomic DNA isolated from the peripheral blood of the two patients using a Roche NimbleGen (Madison, WI) solution-based capture reagent that included the exons of high-confidence protein-coding genes from CCDS, RefSeq, and Vega gene models as well as microRNAs [Ashurst et al., 2005; Pruitt et al., 2007, 2009; Griffiths-Jones et al., 2008]. The library was sequenced with 50-bp reads on the Applied Biosystems SOLiD platform (Carlsbad, CA), and reads were aligned to the March 2006 human reference assembly (NCBI36/hg18). After sequencing, alignment of raw colorspace reads to the human reference assembly was done using BFAST (version 0.6.4d) [Homer et al., 2009], and base quality was then recalibrated using the Genome Analysis Toolkit (GATK) [McKenna et al., 2010]. A mean of 5.3 Gb of sequencing data aligned to the targeted regions of each sample representing 86.8% of bases having ≥10× coverage. Variant calling of Single Nucleotide Polymorphisms (SNPs) and insertions/deletions was done with SAMtools [Li et al., 2009]. Subsequent variants were then filtered using the following criteria: Phred-like variant quality score of at least 40 (or 30 if observed on both strands) and a minor allele frequency of 15%. Common mutations were excluded by filtering high-quality variants against dbSNP130 [Sherry et al., 2001], 1000 Genomes Pilot 1 [Durbin et al., 2010], and a BCM internal database composed of approximately 600 control exomes. We annotated the list of novel mutations for frameshift insertions/deletions, splice-site, missense, and nonsense variants using the ANNOVAR annotation package [Wang et al., 2010]. Functional consequences of nonsynonymous mutations were predicted using the SIFT [Kumar et al., 2009] and PolyPhen-2 [Adzhubei et al., 2010] algorithms.

Causative Mutation Identification

We hypothesized that an X-linked or an autosomal recessive inheritance pattern was responsible for recurrent polymicrogyria in this family given that both patients were male and the first affected members of their pedigree, however, a dominant inheritance mechanism due to germline mosaicism was also a possibility. For the X-linked model we required both brothers to share at least one novel variant in the same X chromosome gene. The parents were of Northern European descent and did not endorse any known consanguinity. As such, we looked for deleterious compound heterozygous mutations shared by both brothers as the most likely explanation for autosomal recessive inheritance. Due to the differences in imaging and in clinical severity in the brothers, we also generated a list of discordant variants not shared by both brothers that may represent genetic modifiers of the PMG phenotype(s).

RESULTS

Exome Sequencing Identifies Compound Heterozygous Mutations in WDR62

The number of shared variants present under different filtering criteria and inheritance models are demonstrated in Table I. Using the X-linked model, we found a single novel shared variant at chrX:77,800,349 in exon 2 of *ZCCHC5* (NM_152694.2), a small two-exon gene encoding 475 amino acids. This missense mutation predicts p.Glu75Asp (c.225G>C) and is expected to be benign by the PolyPhen-2 algorithm. The function of *ZCCHC5* is not well known, and the protein has not been associated with known neuronal development

pathways. Furthermore, a recent study involving sequencing of the coding exons of the X chromosome in families with X-linked mental retardation identified recurrent, common mutations in *ZCCHC5* [Tarpey et al., 2009]. Since common variants are less likely to be deleterious, the identified mutations in *ZCCHC5* were not interpreted to be disease-causing.

Under an autosomal recessive model, we identified three genes with compound heterozygous variants common to both probands: *ZNF717, EPB41L4A*, and *WDR62*. There were two frameshift insertions in exon 5 of *ZNF717* (NM_001128223.1) at chr3:75,869,245 and chr3:75,870,842 that predict p.Cys740SerfsX4 (c.2219_2220insCA) and p.Thr208AspfsX5 (c.622_623insG), respectively. Both mutations are expected to cause early protein truncation. However, we excluded *ZNF717* because it exhibits a high-frequency of nonsynonymous mutations in our internal control database, a finding which suggests that *ZNF717* is not a highly evolutionarily conserved gene. We also identified compound heterozygous mutations in *EPB41L4A* (NM_022140.3). The first mutation at chr5:111,528,715 (c.1933-2_1933-1insATTTT) was located in the 3′ splice site upstream of exon 23 and changes the AG acceptor site to a TG, possibly causing exon skipping. The second event was a missense mutation at chr5:111,629,882 in exon 5 encoding p.Arg127His (c.380G>A) predicted to be deleterious by Polyphen-2. The *EPB41L4A* gene encodes an erythrocyte membrane protein that has no known role in neurogenesis or cortical development, and therefore it was also excluded.

The remaining candidate gene *WDR62* (MIM: #600176) was recently implicated in cortical malformations occurring in consanguineous kindreds via an autosomal recessive inheritance mechanism [Bilguvar et al., 2010; Nicholas et al., 2010; Yu et al., 2010]. Affected individuals in those pedigrees were homozygous for deleterious mutations in *WDR62* whereas the brothers in our study shared compound heterozygous mutations in the gene (Fig. 2A). The first shared mutation at chr19:41,273,990 in exon 17 of *WDR62* (NM_173636.4) predicts p.Ser696AlafsX4 (c.2083delA) while the second shared mutation at chr19:41,283,492 in exon 23 predicts p.Gln918GlyfsX18 (c.2472_2473delAG). Both deletions are expected to cause nonsense mediated decay resulting in reduced or null protein activity. Sanger sequencing validated the probands' mutations and demonstrated that the parents were unaffected carriers of the 1-bp (mother) and 2-bp (father) deletions (Fig. 1G).

DISCUSSION

This study represents the first report of novel compound heterozygous mutations in the *WDR62* gene as a cause of autosomal recessive polymicrogyria. The *WDR62* gene on chromosome 19q13.12 contains 32 exons and normally encodes 1,523 amino acids. Its exact function is unknown, but several studies have suggested an important role in neuronal proliferation and migration [Bilguvar et al., 2010; Nicholas et al., 2010; Yu et al., 2010]. One such study noted increased *WDR62* expression during early mouse brain development specifically in neural crest cells and ventricular and subventricular zones [Bilguvar et al., 2010]. The latter cortical regions are especially important to neurogenesis [Lim and Alvarez-Buylla, 1999]. Proper cortical development depends on the coordinated processes of neuronal proliferation and differentiation, and defects can result in a number of brain malformations [Squier and Jansen, 2010].

A recent report proposed that *WDR62* acts as a spindle pole protein required for proper neuroprogenitor cell division [Nicholas et al., 2010]. Mutations in *WDR62* may therefore interrupt this process leading to abnormal cortical development. Similarly, mutations in another spindle pole gene *ASPM* cause autosomal recessive primary microcephaly (MCPH5; MIM: #608716), which has features overlapping with PMG [Fish et al., 2006; Hutchins et al., 2010]. Mutations in another gene *GPR56* cause bifrontoparietal

polymicrogyria (BFPP), and expression of *GPR56* is required for structural integrity of the pial basement membrane during neuronal migration [Bahi-Buisson et al., 2010]. Thus, it is likely that defects in other structural proteins essential to cortical development also lead to a wide array of brain malformations including PMG.

WDR62 mutations are associated with a wide spectrum of cortical malformations in addition to PMG. Affected individuals often have variable levels of cortical thickening and structural defects that may include the hippocampus and cerebellum and involve one or both brain hemispheres [Bilguvar et al., 2010]. One report suggested that such phenotypic variability may be due to reduced protein function caused by missense mutations versus complete absence of function resulting from nonsense mutations [Nicholas et al., 2010]. However, despite identical frameshift mutations causing premature stop codons in *WDR62*, patient II-1 in our study had a more severe brain malformation characterized by extensive bilateral PMG, an abnormal corpus callosum, and a much more severe clinical phenotype compared to patient II-2who had asymmetric PMG and was less severely affected clinically. Furthermore, no detectable genomic copy number variation was present in either patient to explain their phenotypic differences. We propose several explanations for the phenotypic discordance.

The pregnancy resulting in patient II-1 was complicated by gestational diabetes. Infants of diabetic mothers are at significant risk for major neurologic anomalies including anencephaly, arrhinencephaly, microcephaly, holoprosencephaly, and neural tube defects [Becerra et al., 1990; Tyrala, 1996]. It is possible that the abnormal metabolic environment during fetal development superimposed on the *WDR62* mutations caused the more severe phenotype in patient II-1.

Another possibility is that variation in other genes involved in cortical development could have a synergistic effect on patient II-1's phenotype. Of the 85 rare, non-benign variants unique to patient II-1, we identified two that may have a role in brain development. The first was a novel heterozygous missense mutation at chr2:121,444,473 in *GLI2* (NM_005270.4: c.880G>A) predicting a p.Val294Met change that was not observed in control exomes (Fig. 2B). This gene has been associated with dominantly inherited pituitary anomalies and holoprosencephaly through its role in the developing ventral brain and facial structures [Roessler et al., 2003]. GLI2 is a transcription factor that mediates Sonic Hedgehog signaling in vertebrates and like patient II-1, carriers of *GLI2* mutations may have severe structural brain abnormalities, epilepsy, and developmental delay. Unlike patient II-1, however, pituitary anomalies and polydactyly are often observed with *GLI2* loss-of-function mutations [Roessler et al., 2003]. Patient II-1 (but not patient II-2) also carried a heterozygous mutation at chr10:118,651,341 in *KIAA1598* (NM_001127211.1: c.1598C>T) predicting a p.Pro533Leu change (Fig. 2C). This gene is highly expressed in the brain and encodes a protein with a role in axon guidance via its interaction with actin filament retrograde flow and L1-CAM [Shimada et al., 2008]. To date however, mutations in this gene have not been associated with any clinical phenotypes. Sanger sequencing showed that both the *GLI2* and *KIAA1598* variants were inherited from the phenotypically normal father which decreases the likelihood that they contributed significantly to patient II-1's condition. Further work is required to understand the precise function of *WDR62* in brain development and how other genetic and/or non-genetic factors affect the spectrum of phenotypes observed in patients with *WDR62* alterations.

In conclusion, we report novel compound heterozygous mutations in *WDR62* causing autosomal recessive polymicrogyria in a non-consanguineous kindred. These results support sequence analysis of *WDR62* in the clinical diagnostic testing of individuals with PMG, as

families with an affected child in whom both parents are carriers will have a25%risk of recurrence; and pre-implantation genetic diagnosis is possible.

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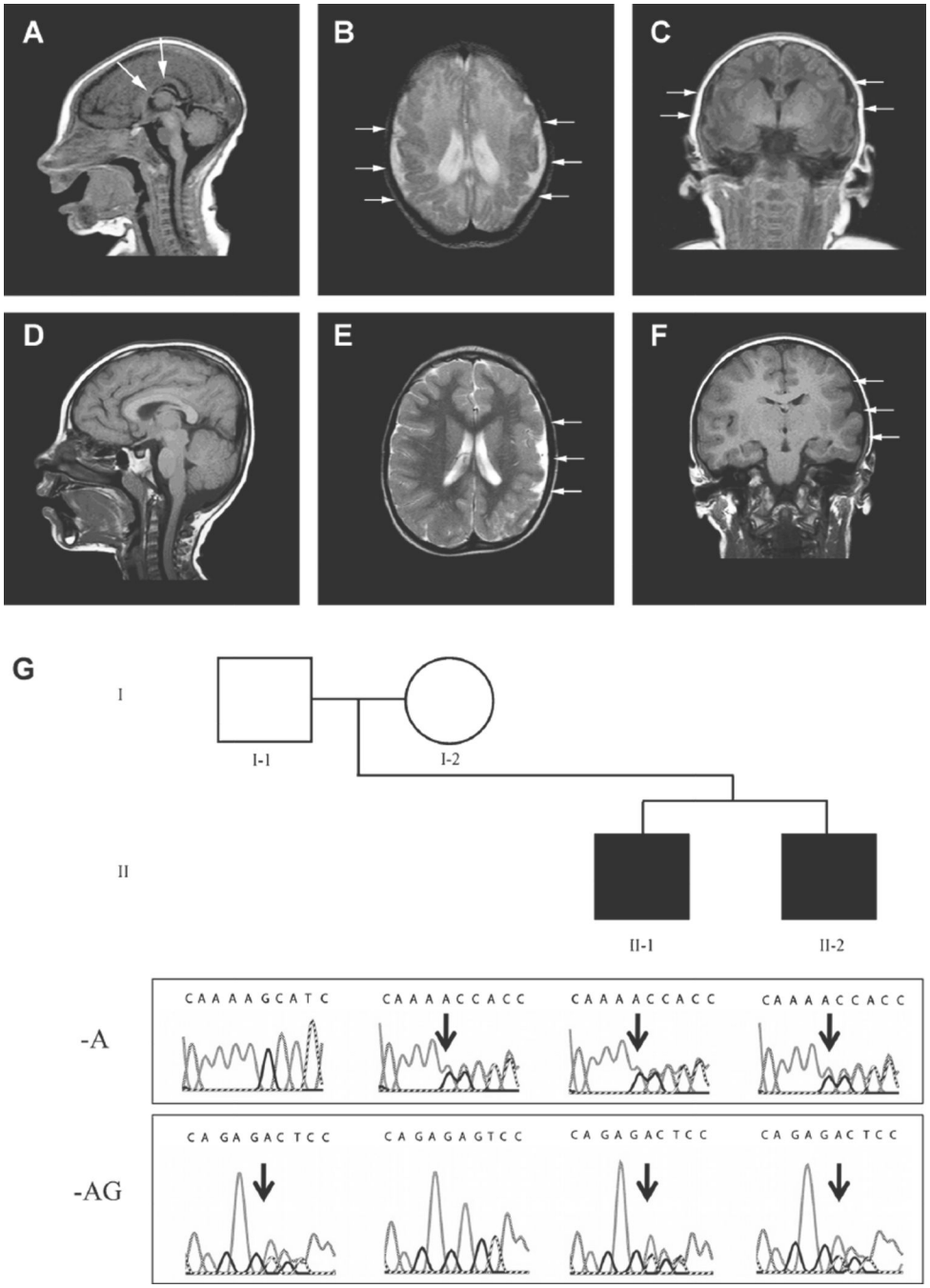

FIG. 1.

Magnetic resonance imaging of the head in patients II-1 (A–C) and II-2 (D–F). A: Sagittal T1 image demonstrates microcephaly and an abnormal corpus callosum (depicted by white arrows). B: Axial T2 and [C] coronal T1 images demonstrate bilateral parietal polymicrogyria and an abnormal sulcal and gyral pattern. Patient II-2: D: Sagittal T1 image demonstrates overall preservation of midline structures. E: Axial T1 and (F) coronal T2 images demonstrate extensive polymicrogyria in the left cerebral hemisphere. White arrows in panels B, C, E, and F depict regions of polymicrogyria. G: Pedigree with Sanger traces below demonstrating *WDR62* mutation family segregation. The affected patients (black squares) inherited both the maternal c.2083delA and paternal c.2472_2473delAG alleles. In

the traces, black arrows show the beginning of each deletion. Following each heterozygous deletion there are overlapping traces representing the wild-type and shifted sequences. [Color figure can be seen in the online version of this article, available at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1552–4833]

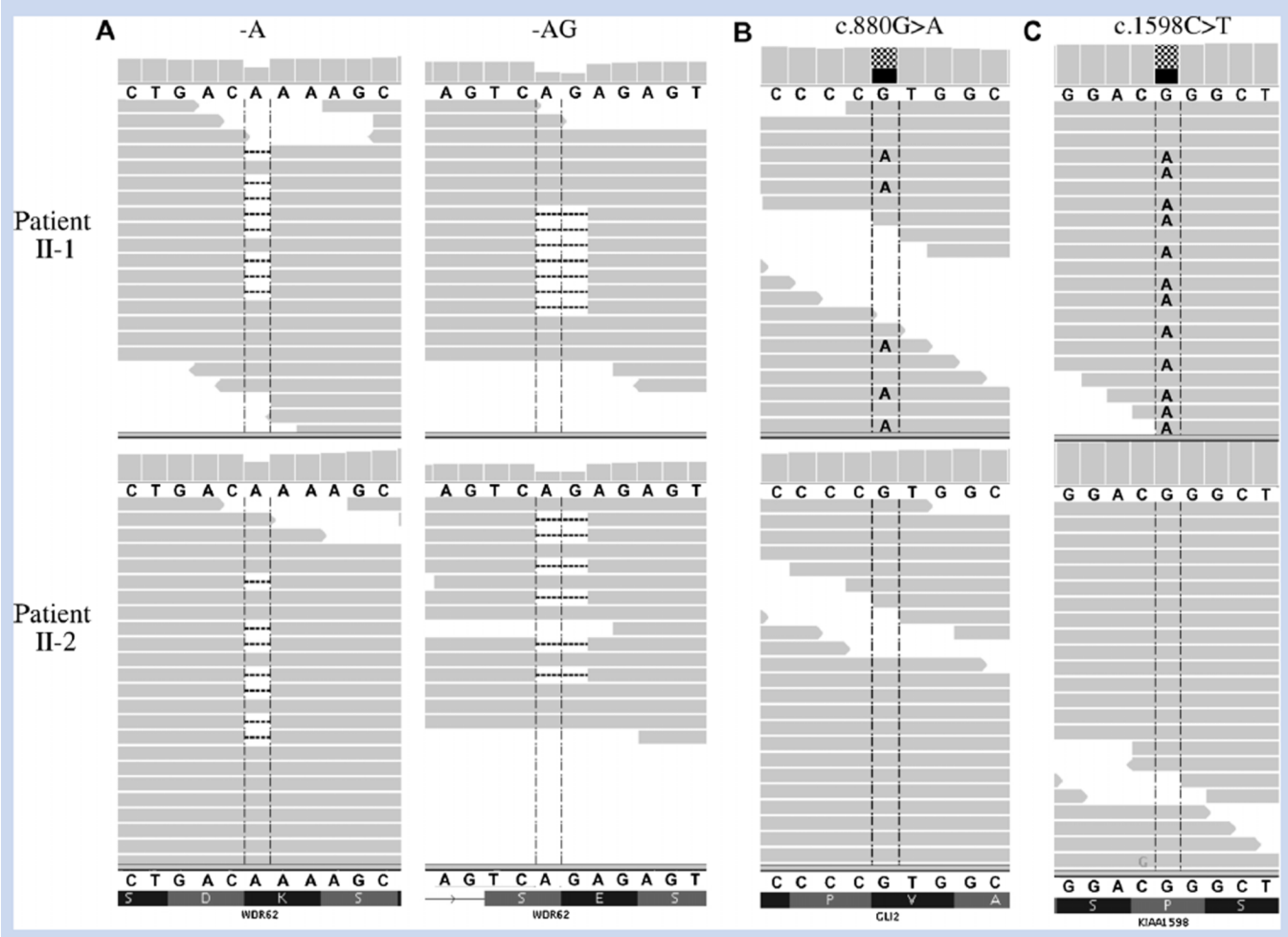

FIG. 2.

A: Sequence alignment visualization showing heterozygous *WDR62* mutations in both patient II-1 (upper panel) and patient II-2 (lower panel). Individual sequencing reads are represented by the horizontal grey bars. Both c.2083delA and c.2472_2473delAG deletions are present in approximately half of each patient's reads, representing a heterozygous state for each mutation. Alignment also shows heterozygous missense variants in (B) *GLI2* and (C) *KIAA1598* in patient II-1 but not in patient II-2. [Color figure can be seen in the online version of this article, available at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1552–4833]

TABLE I

Total and Shared Variants in Siblings with PMG

Nucleotide variants	Patient II-1	Patient II-2	Shared
Coding	17,449	17,264	13,387
NS/SS/I	8,571	8,439	6,411
Rare, non-benign[a]	191	199	106
X-linked	1	3	1
Compound heterozygous	4	7	3

[a]Rare, non-benign variants were not in dbSNP130, 1000 Genomes Pilot 1, or the BCM internal database and were predicted to be deleterious by the SIFT algorithm.

NS, nonsynonymous; SS, splice-site; I, frameshift insertion/deletion.