European Journal of Medical Genetics xxx (xxxx) xxxx

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: www.elsevier.com/locate/ejmg

Identification of a novel *PCNT* founder pathogenic variant in the Israeli Druze population

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ARTICLE INFO

Keywords:
Microcephalic primordial dwarfism
PCNT
Founder
Druze

ABSTRACT

Majewski Osteodysplastic Primordial Dwarfism type II (MOPDII) is a form of dwarfism associated with severe microcephaly, characteristic skeletal findings, distinct dysmorphic features and increased risk for cerebral infarctions. The condition is caused by bi-allelic loss-of-function variants in the gene PCNT. Here we describe the identification of a novel founder pathogenic variant c.3465-1G > A observed in carriers from multiple Druze villages in Northern Israel. RNA studies show that the variant results in activation of a cryptic splice site causing a coding frameshift. The study was triggered by the diagnosis of a single child with MOPDII and emphasizes the advantages of applying next generation sequencing technologies in community genetics and the importance of establishing population-specific sequencing databases.

1. Introduction

The application of next generation sequencing (NGS) technologies in community genetics clinics significantly improved our ability to diagnose rare genetic diseases. There are numerous advantages to the identification of the genetic etiology of rare disease: the specific diagnosis can alter case management and follow up; it provides an end to the diagnostic odyssey; it facilitates family planning and counseling, and triggers research on the disorder. This is especially important in populations with a high burden of homozygous recessive disorders due to consanguineous unions or endogamy. The study of such populations can also lead to the identification of founder pathogenic variants in different ethnic subgroups. Knowledge relevant to such population specific variants can further aid in simplifying the diagnostic process and implementing preconception carrier screening.

Majewski Osteodysplastic Primordial Dwarfism type II (MOPDII) [MIM: 210720] is a form of dwarfism associated with severe microcephaly, characteristic skeletal findings, distinct dysmorphic features and increased risk for cerebral infarcts (Brancati et al., 2005; Hall et al., 2004). Brain imaging reveals evidence of moyamoya disease and/or intracranial aneurysms in 25–50% of the cases (Bober et al., 2010; Brancati et al., 2005; Hall et al., 2004). Intelligence is reported to be borderline or in the low normal range; however, cerebrovascular events can result in cognitive impairment and cerebral palsy (Rauch, 2011; Willems et al., 2010). MOPDII is caused by bi-allelic pathogenic variants in the *PCNT* gene [MIM: 605925], which encodes Pericentrin, a large coiled-coil protein involved in centrosome function and spindle assembly (Rauch et al., 2008). The HGMD and ClinVar databases list over 40 different pathogenic variants in *PCNT*, most are loss-of-function variants. Here we report on the identification of a novel founder splice

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https://doi.org/10.1016/j.ejmg.2019.03.007
Received 5 August 2018; Received in revised form 28 February 2019; Accepted 21 March 2019

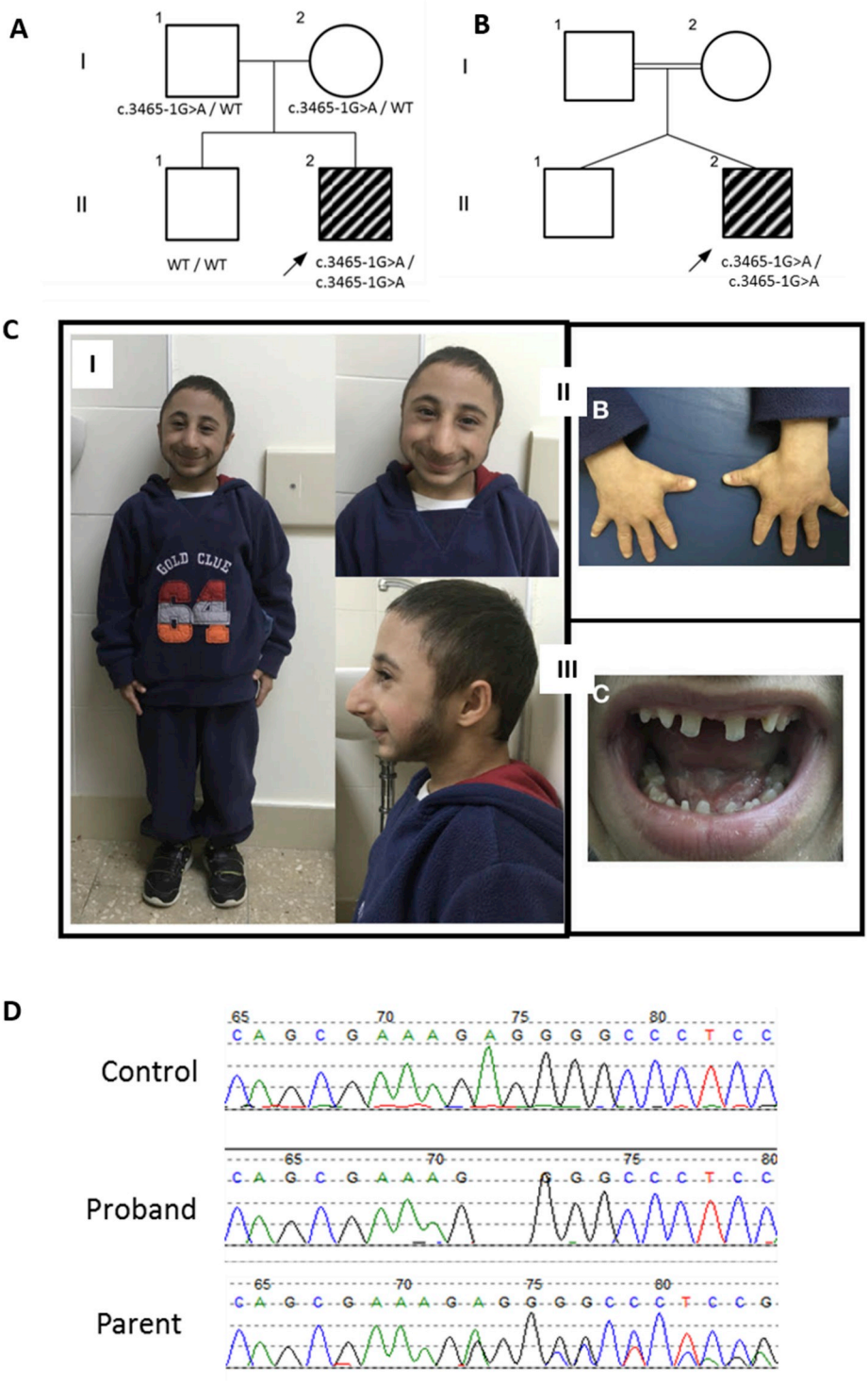

Fig. 1. A: Family 1 pedigree and segregation of the c.3465-1G > A variant: Both parents are carriers, the proband is homozygous and his unaffected brother is a wild-type. B: Family 2 pedigree. C: Photographs of proband 2. I: The final height is 93 cm, the facial features include a prominent nose with broad nasal bridge and long columella below alae nasi, full cheeks and micrognathia. In profile: absence of a sloping forehead, simple pinna with attached lobes, and nasal prominence. II: Brachydactyly. III: Note the small wide spaced teeth with enamel hypoplasia. D: The effect of the c.3465-1G > A mutation on *PCNT* pre-mRNA splicing: Sanger sequencing of cDNA generated from lymphoblastoid cell lines-derived RNA, identified a homozygous AG deletion at the beginning of exon 18 (NM_006031.5) in the proband; both parents were heterozygous. Functionally, the c.3465-1G > A change is equivalent to c.3465_3466del, which causes a frameshift and premature termination of translation (p.Ala1157Profs*36). Sequencing traces were analyzed with Mutation Surveyor v.5.0.1 (SoftGenetics, LLC).

variant in *PCNT* in the Druze population of Northern Israel. The identification of this pathogenic variant was triggered by a simplex case in a single family, emphasizing another advantage for applying NGS technologies in community genetics and the importance of establishing population-specific sequencing databases.

2. Patient data

Family 1 consented to chromosomal microarray (CMA), whole exome sequencing (WES) and biological assays according to an approved institutional review board (IRB) protocol (clinicaltrials.gov: NCT01294345). Family 2 consented to clinical testing of *PCNT* at the Ramabm Genetics Institute (RGI) and for publication of photographs. Screening of 150 paternal Druze households from Northern Israel was performed using a previously established repository (Shlush et al., 2008).

3. Methods

3.1. Genetic testing

For family 1, CMA was performed on the proband using CytoSure

4 × 180 (Oxford Gene Technology). WES was performed at the NIH Intramural Sequencing Center (NISC) using the SeqCap EZ Exome version 3.0 capture kit (Roche NimbleGen) and the Illumina HiSeq2500 platform. Sequencing data were aligned to the GRCh37/hg19 human reference genome using Novoalign (Novocraft), and variants were called using the in-house Most Probably Genotype (MGP) caller. For the proband from family 2 sequencing was performed on the Illumina TruSight One targeted sequencing panel and the NextSeq500 sequencer. Alignment to GRCh37/hg19 human reference genome, variant calling and analysis was done using the Genoox Ltd. data analysis platform (Tel Aviv, Israel). Sanger confirmation and co-segregation analysis were performed for the *PCNT* variant using primers for exon 18 forward: 5′-GAGGTGTGCAAACTGGTGG-3′ and reverse: 5′-AATACGG AGGCTCCTCTCAG-3'.

3.2. Cell culture and RNA isolation

Lymphoblastoid cell lines (LCL) were generated from the blood of the proband in family 1 and his parents using standard procedures. Total RNA extraction from LCLs was performed using a combination of TRIzol® Reagent and the PureLink RNA Mini Kit (Thermo Fisher Scientific) according to the manufacturer's instructions.

3.3. Analysis of the effect of detected variant on mRNA splicing

In order to determine the effect of the *PCNT* variant on mRNA splicing, we generated cDNA from the proband and parents' total lymphoblastic RNA, using the High Capacity cDNA Synthesis Kit (Thermo Fisher Scientific). Primers on flanking exons 17 and 19 (forward primer: 5′-TCCGAGTTGGAGGTGCTG-3′, reverse primer:5′- AGG GCCATCTCCATGAGG-3′) were used for PCR amplification. We used cDNA generated from a SH-SY5Y human neuroblastoma cell line (ATCC) as a control.

3.4. Carrier screening in the Druze population

The samples were screened by restriction fragment length polymorphism analysis using the restriction enzyme Hpy188I according to manufacturer's instructions (New England BioLabs). Hpy188I cleaves the wild type sequence TCAGA but not the mutated sequence TCAAA. After carrier identification using enzyme restriction, we confirmed their genotype with Sanger sequencing as described above.

4. Results

4.1. Patient descriptions

The proband in family 1 was the second child of a couple of Druze descent (Fig. 1A). The parents reported no consanguinity; however, their families originate from the same village. Prenatal sonographic evaluations were notable for severe microcephaly and growth delay. At23 weeks of gestation the length and head circumference were compatible with 19–20 weeks of gestation. The family chose not to perform genetic testing during the pregnancy. The child was born at 37 weeks at birth weight 1440 g (-4SD) with proportionate head circumference (27.6 cm, -4SD). He had dysmorphic features, including a prominent nose, long columella, and a small jaw. He was admitted to the neonatal intensive care unit for low birth weight and nasogastric tube feeding. He had poor weight gain, with a normal swallow test and was subsequently switched to oral feedings. An echocardiogram demonstrated a small membranous ventricular septal defect. The skeletal survey performed at 8 months demonstrated bowing of the arms and smaller than expected ossification centers. At 14 months, the head circumference was 37 cm (−5.5 SD), length 55.8 cm (−5.5 SD), and weight 3.87 kg (−4 SD). He had motor developmental delay; he did not sit or stand on his own but did play with toys and said a few words. Brain MRI performed at 2 years of age demonstrated delayed myelination and widening of the spheno-occipital suture. At the age of 32 months he was hospitalized with the diagnosis of a right frontal stroke and left hemiparesis. He was started on acetylsalicylic acid, and received physical therapy with gradual improvement. On angiography there were three small middle cerebral artery aneurysms. On his most recent examination at the age of 40 months he walked independently and had minimal left-side weakness with pyramidal signs. In addition, there were skin hypopigmentation and malformed teeth. A developmental assessment identified speech and fine motor delays with a Developmental Quotient (DQ) of 55/59 on CAT/CLMAS testing. The patient was referred for genetic testing at 24 months before the diagnosis of cerebral aneurysms. Primordial dwarfism was suspected but not a specific type, and he underwent chromosomal microarray and exome sequencing.

The proband from family 2 was a 16.5 years old male, one of dizygotic twins (Fig. 1B). His parents are first degree cousins of Druze descent. Prenatal screenings were notable for severe intrauterine growth retardation. A karyotype was performed during the pregnancy and was 46XY. He was born via Cesarean section at 32 weeks. His birth weight was 606 g (−5 SD, according to twin growth curves; discordant twins – sibling birth weight 1818 g). He underwent bilateral inguinal hernia repair and orchidopexy. An echocardiogram demonstrated a patent ductus arteriosus (PDA) that was closed with medical treatment. A skeletal survey at the age of 3 years demonstrated delayed bone age, mild kyphoscoliosis and genu varum. He was diagnosed with global developmental delay and mild intellectual disability. At 7 years of age, he started growth hormone therapy, which was discontinued a year later due to lack of improvement. Brain MRI performed at 8 years of age demonstrated pachygyria, and magnetic resonance angiography (MRA) of the circle of Willis was normal. On his most recent physical examination at 16 years, his height was 93 cm (−8.8 SD), weight 13.6 kg (−19.8 SD), head circumference 41.7 cm (~-10 SD). He has a high-pitched voice, sociable personality, severe microcephaly, beaked prominent nose with broad nasal bridge and long columella, micrognathia, sharp and translucent teeth, and brachydactyly (Fig. 1C). He had Tanner IV testicular volume of 3 ml (pre-pubertal) with full virilization and areas of hypopigmentation (Pityriasis versicolor like) on his trunk. The patient was clinically suspected to have MOPD type II based on the findings on clinical examination and was referred for *PCNT* sequencing at 16 years.

4.2. Array, sequencing and RNA analysis results

The CMA of proband 1 was negative for pathogenic copy number variations. Large regions of homozygosity (ROH) were identified (~55 Mb) consistent with ~2% identity by descent or a second cousin relationship, which can be explained by endogamy. The candidate genes within the ROH associated with growth delay and microcephaly were *PIGO*, *EXOSC3*, *FAR1*, *PGAP3*, *CDC6* and *G6PC*. However, WES identified a homozygous canonical splice site variant in *PCNT* c.3465-1G > A (NM_006031.5) (ClinVar submission SUB5252415) that fit the patient's phenotype but was not within the ROH detected by array. The c.3465-1G > A variant was reported in four heterozygous individuals from 'South Asia' in the gnomAD database with an allele frequency of 0.0001 in this sub population, and was not present in the Greater Middle East (GME) Variome Project database (Scott et al., 2016). Another heterozygous male carrier was identified in the Rambam Genetics Institute internal dataset, also of Druze descent. Subsequently, we identified the same homozygous splice variant in the proband from family 2 who was referred to our center for *PCNT* sequencing and his family originates from a different Druze village in Northern Israel.

Sanger sequencing analysis of the cDNA from the first proband and his parents revealed a two base pair deletion at the beginning of exon 18 due to the activation of a cryptic splice site (Fig. 1D), causing a coding frameshift (p.Ala1157Profs*36) predicted to result in a

truncated protein lacking coiled coil domains 4, 5 and 6, and the NEK2 binding site.

Screening of 150 Druze DNA samples from different paternal households revealed five heterozygous carriers (1:30, CI 95%[0.004–0.062]). The carriers originated from four different Druze villages in Northern Israel. Based on the NGS data we were able to identify a haplotype shared by the two probands and an unrelated carrier. The haplotype was localized to 450 kb in the sub-telomeric region of 21q22.3. The minimal region was between rs34495634 and rs200348425 and the genomic coordinates: chr21:47409580–47859993.

5. Discussion

MOPD II is a form of primordial dwarfism associated with cerebrovascular disease and a poor prognosis. Here we report on a novel founder mutation in the Israeli Druze population. Both children reported here had a typical clinical presentation with severe pre and postnatal growth delay and one of them had cerebrovascular disease and more significant developmental delays. The variant was detected in the exon 18 acceptor splice site. RNA studies demonstrated that the variant results in the activation of a cryptic acceptor splice site, leading to a coding frameshift and truncation of the PCNT protein. Therefore, RNA studies support the pathogenicity of this variant. Following the diagnosis of the index family we identified another unrelated carrier of Druze decent in our (RGI) internal database of NGS data from Northern Israel. This triggered screening of 150 Druze paternal households, identifying a carrier rate of 1:30. In parallel, we identified another Druze individual with MOPD type II and the same homozygous splice variant in *PCNT*. The affected children and carriers we identified originated from seven different Druze villages in the northern Israel, including the Galilee and Carmel regions, suggesting this variant is a founder mutation in this population and not a private mutation related to a certain village or family. The Druze population is a transnational population, including minority communities in Israel, with a long history of cultural isolation and endogamy (Falik-Zaccai et al., 2008; Mory et al., 2012; Shlush et al., 2008). In Israel the majority of Druze live in 19 villages in the Galilee, Carmel and Golan heights. Several monogenic disorders were found to be frequent in specific Druze villages in Israel (Mory et al., 2012; Shlush et al., 2008), and a few disorders were identified in more than one village (Kalfon et al., 2017) The current Druze population size in Israel is ~140,000 individuals and the number of births per year is ~2400 in the past 10 years (Israeli Central Bureau of statistics http://www.cbs.gov.il, 2016). Interestingly, our center and other centers in Northern Israel were not aware of a high prevalence of MOPD type II in this population. Possible explanations would be the occurrence of simplex cases diagnosed in separate clinical centers, limited resources for large gene sequencing, fetal demise or pregnancy termination. The family has been counselled regarding the recurrence risk and pre-gestational versus prenatal diagnostic options. Furthermore, the identification of a high carrier rate in the Druze population in Israel will lead to the inclusion of this variant in the national preconception screening program (Zlotogora et al., 2016). This report emphasizes another advantage arising from the application of NGS in community genetics and the utility of population-specific NGS data sets.

Acknowledgements

We are thankful to the families for consenting to participate in this publication. The NIH Intramural Sequencing Center (NISC) conducted the sequencing experiment in one of the probands. This work was supported by the intramural program of the National Human Genome Research Institute, NIH. Dr. Karin Weiss is supported by The Clinical Research Institute at Rambam.

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