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SHORT REPORT

CLINICAL GENETICS WILEY

PDCD6IP, encoding a regulator of the ESCRT complex, is mutated in microcephaly

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Abstract

Primary microcephaly (PM) is a highly heterogeneous neurodevelopmental disorder with many contributing risk genes and loci identified to date. We report a consanguineous family with PM, intellectual disability and short stature. Using whole exome sequencing, we identified a homozygous frameshift variant in programmed cell death 6 interacting protein (*PDCD6IP*, c.154_158dup; p.Val54Profs*18). This gene, *PDCD6IP*, plays an important role in the endosomal sorting complexes required for transport (ESCRT) pathway in the abscission stage of cytokinesis and apoptosis, and is required for normal brain development in mice. The clinical features observed in our patient were similar to the phenotypes observed in mouse and zebrafish models of *PDCD6IP* mutations in previous studies. This study provides evidence that clinical manifestations of *PDCD6IP* mutations as seen in our patients with PM and ID may be a novel cause for neurodevelopmental disorders.

KEYWORDS

consanguineous family, intellectual disability, microcephaly, *PDCD6IP*, whole exome sequencing

1 | INTRODUCTION

Microcephaly is a clinically and genetically heterogeneous neurodevelopmental condition that is distinguished by reduced head circumference less than two (≤2) standard deviations (SD) below the mean for sex, age, and ethnicity with mild to severe intellectual disability (ID).[1–3] The overall incidence of microcephaly at birth varies from 1.3 to 150/100 000 in different worldwide populations.[4] Microcephaly is predominantly autosomal recessive (AR) and is therefore more prevalent in consanguineous unions than other family structures.[3,4] However, families with either autosomal dominant or X linked inheritance have been reported.[5,6] Other clinical features

associated with microcephaly are delayed motor and cognitive development, intellectual disability, movement disorders, feeding and vision problems, epilepsy, and autism.[7,8] The phenotypic spectrum of microcephaly and associated disorders is wide, with more than 650 entries in the OMIM (Online Mendelian Inheritance in Man) database[8–10] and more than 20 genes have been identified responsible for AR primary microcephaly.[10,11] These genes are involved in a range of important biological processes overarching cell cycle regulation including, mitotic spindle assembly and structure, centrosome and centriole function, DNA repair and damage response during cell cycle, kinetochore-associated functions, chromatin remodeling complexes, cleavage furrow formation, cytokinesis and midbody regulation.[11,12]

The advent of next-generation sequencing (NGS) technology in the field of genetics has provided an unprecedented opportunity for the identification of rare pathogenic variants causing Mendelian disorders. Recently, 104 Arab families having 150 affected individuals with 56 Mendelian forms of congenital microcephaly (CM) were analyzed for causative variants using different technologies including targeted or exome sequencing, and autozygome analysis.[13] They identified pathogenic variants in known and novel genes and categorized them into four groups; variants in MCPH genes, variants in genes with established disease phenotypes in humans, variants in genes reported previously as candidate genes and variants in genes with no established disease phenotypes in humans.[13] They expanded the genetic heterogeneity with developmental brain defect in Arab populations which are generally underrepresented in large public disease and non-disease variant databases.[13]

Herein, we describe, a consanguineous Saudi family having two affected male individuals (Figure 1A) segregating likely AR primary microcephaly. Whole exome sequencing was used to identify a candidate gene for the disorder supported by common phenotypic features observed in mouse and zebrafish models.[14,15]

2 | PATIENT AND METHODS

2.1 | Human studies

The study design and protocol was reviewed and approved by the Ethical Review Committee (ERC) of King Abdullah International Medical Research Center (KAIMRC, Riyadh, Saudi Arabia), as well as by the King Saud Bin AbdulAziz Health Sciences University. Signed informed consent for the genetic analysis and publication of data was obtained from the patient's legal guardians. Pedigree was drawn (Figure 1A) and the affected individuals were thoroughly examined by a local consultant geneticist and medical doctor. Clinical information including age, gender, family history and consanguinity was recorded. Informed consent to participate in the study was obtained from the parents of participants.

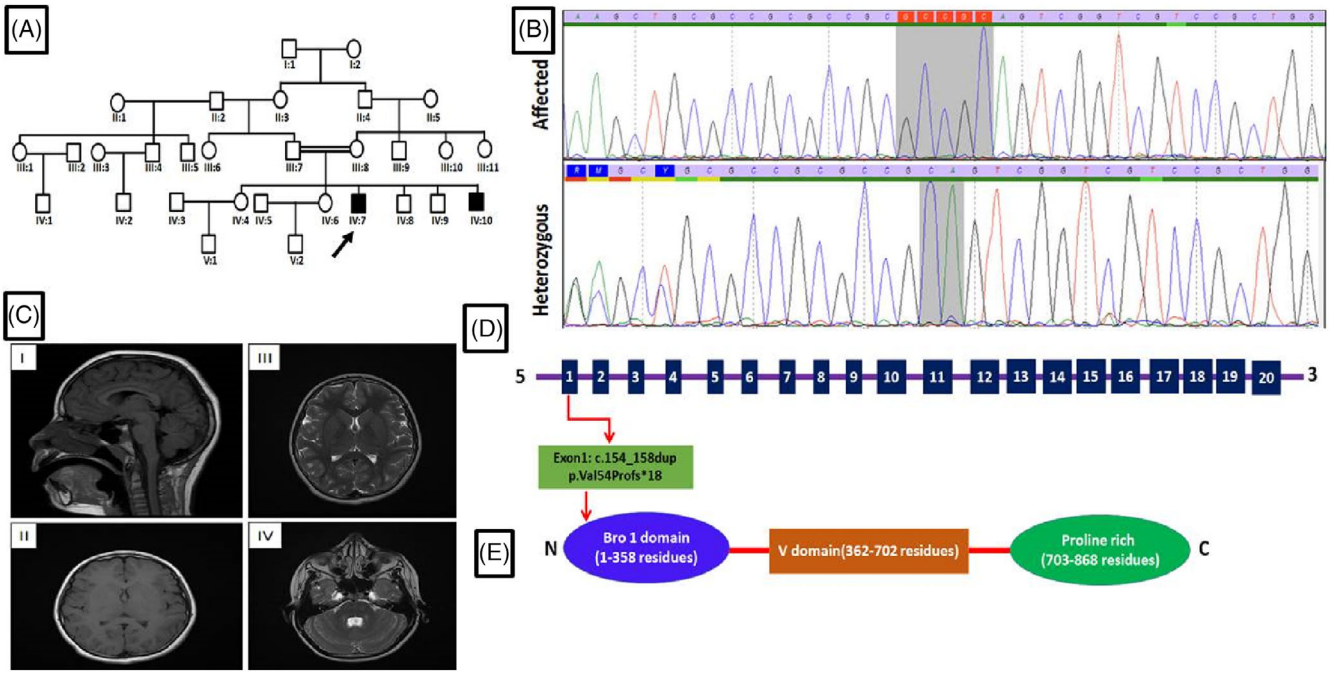

FIGURE 1 A, Pedigree of the present family. B, Sanger sequence chromatograms of the affected individuals (upper panel) or heterozygous carriers (lower panel) with *PDCD6IP* mutations. C, MRI of the proband, Sagittal T1-WI (I) shows microcephaly. Axial T1-WI and T2-WI (II, III) demonstrate a mildly simplified gyral pattern with normal cortical thickness. Axial T2-WI (IV) shows a disproportionally large cerebellum. D, Schematic representation of all 20 exons of the *PDCD6IP* gene. E, Known three structural and functional domains (Bro 1, V and Proline rich) of the mature *PDCD6IP* protein. Arrows show the position of the mutation (c.154_158dup; p.Val54Profs*18) identified in this study [Colour figure can be viewed at wileyonlinelibrary.com]

2.2 | Blood sample collection and DNA extraction

Blood samples were drawn from both affected children and their unaffected parents and DNA was isolated by using commercially available kits (QIAamp, Qiagen, Valencia, CA, USA). DNA quality was analyzed by agarose gel electrophoresis and Nanodrop-2000 spectrophotometer (Thermo Scientific, Schaumburg, IL, USA) was used for DNA quantification.

2.3 | Library preparation and whole exome sequencing

DNA samples of IV: 7 and IV: 10 and their unaffected parents were submitted for whole exome sequencing at Centogene, the rare disease company (https://www.centogene.com). RNA capture baits against approximately 60 Mb of the human exome (targeting>99% of regions in CCDS, RefSeq and Gencode databases) were used to enrich regions of interest from fragmented genomic DNA with Agilent Sure Select Human All Exon V6 kit. The generated library was sequenced on an Illumina platform (Illumina, San Diego, CA, USA) to obtain an average depth of ~100×. Typically, ~97% of the targeted bases were covered >10×.

2.4 | Data analysis

Centogene's end to end in-house bioinformatics pipeline including base calling, alignment of reads to GRCh37/hg19 genome assembly, primary filtering out of low quality reads and probable artifacts, and subsequent annotation of variants was applied. All disease-causing variants reported in the human gene mutation database (HGMD), ClinVar or CentoMD as well as all variants with minor allele frequency (MAF) of less than 0.1% in the gnomAD database were considered. Evaluations were focused on coding exons along with flanking +/−20 intronic bases. All pertinent inheritance patterns (AR, X-linked and de novo) were considered. In addition, provided family history and clinical information were used to evaluate eventually identified variants. All identified variants were evaluated with respect to their pathogenicity and causality. All variants related to the phenotype of the patient, except benign or likely benign variants, were reported (Supporting Information Tables S1 and S2). Variants of relevance which met Centogene internal quality control (QC) criteria (based on extensive validation processes) were not validated by Sanger sequencing. The *PDCD6IP* gene was analyzed by polymerase chain reaction (PCR) and bidirectional sequencing of both DNA strands of the relevant coding region (Figure 1B).

3 | RESULTS

3.1 | Clinical report

A consanguineous family with two affected male individuals in one generation (Figure 1A) was ascertained for the presence of microcephaly and intellectual disability (ID) from the kingdom of Saudi Arabia. The two children (IV: 7 and IV: 10) were diagnosed at department of Pediatrics, King Abdullah Specialized Children's Hospital (KASCH) Riyadh, Saudi Arabia. In both children, primary microcephaly was noticed at birth and their occipital-frontal circumference (OFC) was ≤−2 standard deviations (SD) (www.who.int/childgrowth/standards), with a likely AR mode of inheritance. Detailed information including the parental marriage type, pedigree, disease history, affected and non-affected subjects and a number of sibships was obtained by interviewing family elders. The affected individuals (IV: 7 and IV: 10) had microcephaly and ID, disproportionate short stature, atrophic non-functional right kidney and developmental delay (speech delay, with cognitive impairment). There was no history of any neurological disease in the family. They were unable to do simple tasks, self-care, and had poor communication skills. Their learning abilities were substantially limited, however, the proband (IV: 7) is currently at special education school. On physical examination at 19 years of age, the proband IV: 7 weight was 52 kg (−2.0 SD), height 132.08 cm (−6.2 SD), and head circumference 48.5 cm (−4.4 SD), below the population age and sex-related mean. He has mildly short forehead, deep-set eyes, thick eyebrows, hypoplastic philtrum and mildly pointed chin. Social and behavioral abnormalities, ID, epilepsy, and strabismus are noted. However his hearing, heart and limb anomalies were observed normal. An MRI of the proband (IV: 7) brain at age of 18 showed small craniofacial ratio on sagittal T2WI image which indicate microcephaly. Mildly simplified gyral pattern was noted on Axial T1WI image. The corpus callosum was normal in volume and signal intensity. The posterior fossa structures showed normal brain stem with relatively large cerebellum (Figure 1C). Radiological findings of the kidney revealed echogenic, an atrophic, small-sized right kidney measuring approximately 2.5 cm, with poor corticomedullary differentiation. The left kidney measured 8.6 cm with preserved corticomedullary differentiation. There were no other focal lesions or hydronephrosis. The urinary bladder was not fully distended but within normal limits.

The proband brother (IV: 10) is 10 years old, who presented the same symptoms of disease including high forehead, thick eyebrows, and deep-set eyes with a squint. At the time of physical examination, his age was 10 years, weight 19.5 kg (−3.0 SD), height 106.68 cm (−5.0 SD) and head circumference 47 cm (−4.5 SD). Microcephaly, ID, early onset epilepsy, social, and behavioral abnormalities were noted. Detailed physical assessment revealed normal hearing, nose, mouth, and scalp hair (Table 1). Chromosome analysis revealed an apparently normal male karyotype (Karyotype: 46, XY). Details clinical information is summarized in Table 1.

3.2 | Molecular analysis

Across the 22 autosomes, only one homozygous, coding, frameshift duplication variant in the programmed cell death 6 interacting protein (*PDCD6IP*) gene NM_001162429.2; c.154_158dup;(p.Val54Profs*18) was identified that segregated in both affected individuals (Figure 1B)

TABLE 1 Clinical features of the affected individuals

Parameter	Subject (IV: 7)	Subject (IV: 10)
Sex	Male	Male
Age at last examination (years)	19	10
Ethnicity	Arab, KSA	Arab, KSA
Consanguinity	Yes	Yes
Microcephaly	+	+
Head circumference (cm, SD)	48.5 (−4.4)	47 (−4.5)
Weight at last examination (kg, SD)	52 (−2.0)	19.5 (−3.0)
Height (cm, SD)	132.08 (−6.2)	106.68 (−5.0)
Developmental delay	+	+
Age of walking (months)	14	16
Intellectual disability	+	+
Mood instability	+	+
Schooling	+	–
Ataxia	–	–
Tic disorders	–	–
Congenital hearing loss	–	–
Eye anomalies	Strabismus	Strabismus
Language articulation	–	–
Hyperactivity	+	+
Cardiac anomalies	–	–
Limb anomalies	–	–
Hypertonia/Hypotonia	–	–
Skin anomalies	–	–
Renal findings	Kidney atrophy	–
Abdomen	–	–
Seizures	+	+
Lung disease	–	–
Pregnancy event	Normal	Normal
Endocrine problems	–	–

Abbreviations: KSA, Kingdom of Saudi Arabia, +, present, –, absent.

and was not found in dbSNP (http://www.ncbi.nlm.nih.gov/SNP), Exome Variant Server (EVS, http://www.evs.gs.washington.edu/EVS),1000 genome project (http://www.1000genomes.org), ExAC (http://exac.broadinstitute.org), or the gnomAD (https://gnomad.broadinstitute.org) databases. The *PDCD6IP* variant occurs at a highly conserved residue, and is predicted to be deleterious in all available bioinformatics results (Table 2). This variant c.154_158dup (p.Val54-Profs*18) occurs in the first exon of *PDCD6IP* (Figure 1D). The resulting protein if expressed would be predicted to be non-functional. This homozygous variant was confirmed to be present in both affected children (IV: 7 and IV: 10), while the four healthy siblings (IV: 4, IV: 6, IV: 8 and IV: 9) and parents (III: 1 and III: 2) were heterozygous for the wild type allele as shown in the Figure S1.

TABLE 2 Homozygous variant on chromosome 3 from exome data of the family

Family	1
GRCh37/hg19 (2009) position	Chr3:33840063-33 911 199
Genomic size	71 137
Genomic region	3p22.3
Reference allele	GCCGC
Alternate allele	GCCGC
Gene symbol	*PDCD6IP*
MIM number	608 074
GenBank	001162429.2
Total exon	20
cDNA change	c.154_158dup
Chromosome position	33 840 379
Amino acid change	Val54Profs*18
Location	Exon 1
Variant type	Insertion
PolyPhen2	N/A
SIFT	N/A
Mutation Taster	1/Damaging
GnomAD	–
ExAC_hom	–
1000G	–
HGMD	–
Exome Sequencing Project (ESP)	–
CentoMD	–
ACMG classification	PVS1
Segregates with phenotype	Yes

Abbreviations: HGMD, human genomic mutation database; N/A, not applicable; PVS1, pathogenic very strong.

4 | DISCUSSION

Programmed cell death 6 interacting protein (*PDCD6IP*), which is also known as ALG2-interacting protein 1 (*ALIX*) is one of the most intensely studied multifunctional cytosolic and multi-domain scaffold proteins.[16] *PDCD6IP* acts at the cell membrane to permit endophilins in clathrin-mediated endocytosis[17,18] and at endosomes to regulate cysteine-aspartic proteases (caspase) activation through binding to proteins of the endosomal sorting complexes required for transport (ESCRT).[19,20] The ESCRT machinery comprises five distinct ESCRT complexes (ESCRT-0, -I, -II, -III and the Vps4 complexes) and several accessory proteins.[21,22] ALIX is an accessory protein interacting with components of both ESCRT-I and ESCRT-III.[23] *PDCD6IP* is ubiquitously expressed[16] and performs multiple functions, including membrane repair, cytokinesis, cell proliferation and abscission, viral budding, as well as exosome secretion and autophagy[24] which all rely on ESCRT-mediated membrane degradation and proliferation.[14] Additionally, the product of *PDCD6IP* gene binds to the apoptosis

promoting protein, PDCD6, in a calcium (Ca^{+2}) dependent manner and plays a role in breast cancer.[23,25] *PDCD6IP* has co-evolved with *MYORG* and encodes a protein that shows strong interaction with PDGFRb (platelet-derived growth factor receptor beta).[27] *PDCD6IP* is expressed in astrocytes and its over expression can inhibit PDGFRb interlization.[27,28] Thus, PDCD6IP/ALIX has manifold cell functions, and the precise, essential functions that are disrupted by the mutations we present here are not known in detail.

In this study, we report a Saudi consanguineous family with primary microcephaly in an AR pattern of inheritance, associated with homozygous *PDCD6IP*, c.154_158 dup; p. (Val54Pros*18). The affected patients in this family presented with microcephaly, deep-set eyes, delayed speech and language, and moderate to severe ID. Our findings are in general agreement with a previous study in mouse, showing that *Pdcd61ip* mutation is associated with microcephaly.[14] A previous study in human also showed that de novo heterozygous deletion of the *PDCD61P* locus is associated with neurological dysfunction, specifically cerebral palsy, although given that this is a single report, the specificity of this CNV association is still somewhat uncertain. Heterozygous carriers in the family we describe here would likely show complete loss of one allele, yet showed no clinical abnormality, which would be markedly different from the previously reported de novo heterozygous deletion in two males that was associated with cerebral palsy,[15] so further study will be needed to understand the effects of heterozygous loss of *PDCD61P*.

Pdcd6ip−/− mice develop severe microcephaly in early embryonic development believed to be due to a substantial, yet transient wave of cell death of neural progenitors in the E11.5 and E12.5 telencephalon causing a lateral reduction of the tissue and loss of inner-layer neurons.[14,26] Subsequent developmental stages appear normal giving rise to newborn cortices with volume reduction but normal cortical layers except for thinner layer VI. Nevertheless, radial expansion during the post-natal period is altered giving rise to an adult cortex with all layers considerably thinner. This alteration is accompanied by defects in neurite extension observed both in vivo and also in vitro.[14,26] These *Pdcd6ip* knockout mouse data suggests that Pdcd6ip plays a pivotal role in brain size determination by controlling neural progenitor cells survival at the start and later of neurogenesis, by regulating post-natal dendrite expansion.[14,26] Zebrafish with knock-down of *pdcd6ip* showed significant effects on movement including hyperactivity and erratic swimming behavior when compared with controls.[15] Other morphological features such as microcephaly, mild, moderate or severe developmental abnormalities, loss of pigment in eye, decreased eye size, gray matter hindbrain, small head, domed cranium, cardiac abnormality, decreased body size and tail curvature or kinking and cardiac oedema were also seen following knock down of the *pdcd6ip* expression in zebrafish compared to the control,[15] suggesting that Pdcd6ip has effects on many cell types in the body. Importantly, we report that homozygous loss of function has significantly more severe clinical outcome than previously reported case of heterozygous loss through whole gene deletion.[15] Overall our findings support that *PDCD6IP* is a dosage-sensitive gene implicated in microcephaly.

5 | CONCLUSION

Here we implicate loss of function of *PDCD6IP* to the etiology of microcephaly and ID in humans. The demonstrated requirement for Pdcd6ip for cell survival and proliferation in mice in cortical development suggests a plausible explanation for microcephaly due to loss of this gene. Damaging variants in *PDCD6IP* should be considered in cases of microcephaly with ID.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Amjad Khan, Manal Alaamery, Salam Massadeh and Abdulrahman Obaid collected samples, clinical data, analyzed data, and performed experiments. Amjad Khan and Manal Alaamery prepared the initial draft of the manuscript. Amjad Khan and Amna A. Kashgari assisted in developing figures, images and table for the manuscript. Christopher A. Walsh and Wafaa Eyaid supervised, supported, and edited the manuscript. All the authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

Any additional data required will be available on request

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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