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Case Report

Multi affected pedigree with congenital microcephaly: WES revealed PNKP gene mutation

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Abstract

Microcephaly is a rare neurological disorder, occurs in both isolated and syndromic forms. This classification could be confusing in rare disorders with variable phenotypic characteristics. However, identification of the causative gene through genetic study would allow determining the definite diagnosis. Here we reported a novel missense variant c.1133A>C (p.Lys378Thr) on the 13th exon of *PNKP* gene identified by whole exome sequencing (WES) in an Iranian multi-affected family with microcephaly, seizures and developmental delay (MCSZ) disorder. Data analysis suggested this variant as a pathogenic mutation which is co-segregate with the disease in the pedigree. *PNKP* gene mutation is consistent with the clinical features of the affected family members. Regarding both genetic findings and clinical examinations, the reported pedigree can be considered as another affected family with MCSZ syndrome, which has been reported about 10 cases worldwide. This study proves the application of WES for determining the final diagnosis in complicated neurodevelopmental disorders.

Keywords: Whole exome sequencing; Microcephaly; *PNKP*; MCSZ syndrome

1. Introduction

Microcephaly can occur alone as isolated microcephaly or in association with other neurological abnormalities as syndromic microcephaly. Classification of syndromic cases can be made based on clinical findings and phenotypic characteristics. However, definite diagnosis in some families especially with milder phenotypes or subclinical manifestations depends on further evaluations, principally genetic investigations [1]. Defects in numerous biological processes are responsible in either isolated or syndromic microcephaly. Up to date more than 600 different genes are related to microcephaly term (HP:0000252) [2] which some of them are identified only in a few families or certain ethnic groups. An example is *PNKP* gene [OMIM * 605610] on chromosome 19q13.33. The protein product is a bifunctional DNA repair enzyme, which was introduced since 2010 to be responsible for a rare syndromic neurodevelopmental

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disorder called microcephaly, seizures and developmental delay (MCSZ) (OMIM #613402). Further studies described notable phenotypic variety among reported affected families from severe microcephaly and severe tonic–clonic convulsions to moderate microcephaly with seizures controlled by medication, associated with variable other neurodevelopmental symptoms. Up to date it has been recorded about 10 families worldwide [3–6].

Here, with the help of NGS technology, we aimed to identify the causative gene in a three-generation Iranian family having six affected individuals with predominant phenotype of microcephaly and moderate developmental delays.

2. Patients and methods

2.1. Case report

A consanguineous family having a 13-year boy with congenital microcephaly and developmental delay, from north-east of Iran was referred for clinical and genetic evaluation.

The proband boy was born at term via cesarean section (due to circulating umbilical cord) from a 30-year-old mother. During pregnancy, she has experienced pregnancy nausea. The parents were first cousins. His Apgar scores and neonatal growth indexes were normal at birth except for congenital microcephaly (Weight: 2800 g, Head circumference: 32 cm, Length: 50 cm). His growth milestones were within the acceptable range for weight and length but were abnormal for the head circumference. He has moderate gastroesophageal reflux that was finally controlled by using special amino-acid formula (Nutramigen). His developmental milestones especially the speech axis were moderately delayed. Neurological examination showed a mild to moderate central hypotonia and moderate global developmental delay. The brain magnetic resonance imaging (MRI) showed cerebellar atrophy. The funduscopic examination demonstrated mild optic atrophy. He also experienced seizure at 4 years of age which was controlled by sodium valproate and ethosuximide. The Electrodiagnostic examination shows absent SNAPs and mildly reduced CMAPs in lower limbs. Metabolic screening by tandem mass spectroscopy on dried blood spot and evaluation of organic acids in urine were normal.

Further genetic counseling revealed the remarkable history of congenital microcephaly with another 5 affected members ranging from 3-year old to 30-year old within two generations (Fig. 1A). Brain MRI (axial T1, T2 FLAIR Sag T2 MRI without contrast media) findings of individual VI:5 showed normal results at the age of 2 years old, with unremarkable the sella, pituitary and parasellar structures. Also the cerebellopontine angles, brain stem, cerebellar hemispheres, petromastoid regions and orbits are unremarkable. Additional information about the affected individuals was summarized in Table 1.

2.2. Genetic studies

Peripheral whole blood samples were collected from all available members of the family, totally 27 individuals, after proper genetic counseling. Informed consent was obtained from all adult members and patient's parents. This study was approved by the Research Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran.

Targeted NGS panel was performed to analyze 10 related genes for primary congenital microcephaly including *MCPH1*, *WDR62*, *CDK5RAP2*, *CEP152*, *CENPJ*, *STIL*, *SLC25A19*, *ASPM*, *CASC5* and *CEP135* by BGI, Dx, Hong Kong.

WES was performed for the VI:3 patient as the proband. DNA capturing and paired-end sequencing with 100× coverage were performed by Macrogen company (South Korea). Genomic DNA was captured on Agilent SureSelect V6 Target Enrichment Kit and sequenced with Illumina HiSeq 4000 platform.

The stepwise approach was performed for data analysis and an in house filtering process was done to identify the causative mutation. dbSNP, 1000 Genomes database, 6503 exomes from NHLBI GO Exome Sequencing Project (http://evs.gs.washington.edu/EVS/) and ExAC database (http://exac.broadinstitute.org/) were used for population frequency filtering. According to variant effect's, synonymous, upstream/downstream and intronic variants except the 20–30 bp flanking each exons, were excluded from analysis. PubMed and OMIM were reviewed for prioritize the genes related to microcephaly and encephalopathy. Bioinformatic investigations to evaluate the potential effect and pathogenicity of candidate variants were conducted by online tools including SIFT, Provean, PolyPhen2, Mutation Taster and Combined Annotation Dependent Depletion (CADD), as well as conservation predictive tools such as PhyloP and PhastCons score.

Sanger sequencing was used to validate the identified variant in the proband. Also segregation analysis was performed on DNA samples of 5 affected relatives and 21 available healthy individuals of the family.

3. Results

No causative mutation found in any of the 10 genes analyzed by targeted NGS. WES identified a total number of ~82 M reads, while 78% of reads mapped to 50 Mb target regions. Among the candidate variants only the novel missense variant c.1133A>C (p.Lys378Thr) in the 13th exon of *PNKP* gene (NM_007254.3) was compatible to the proband.

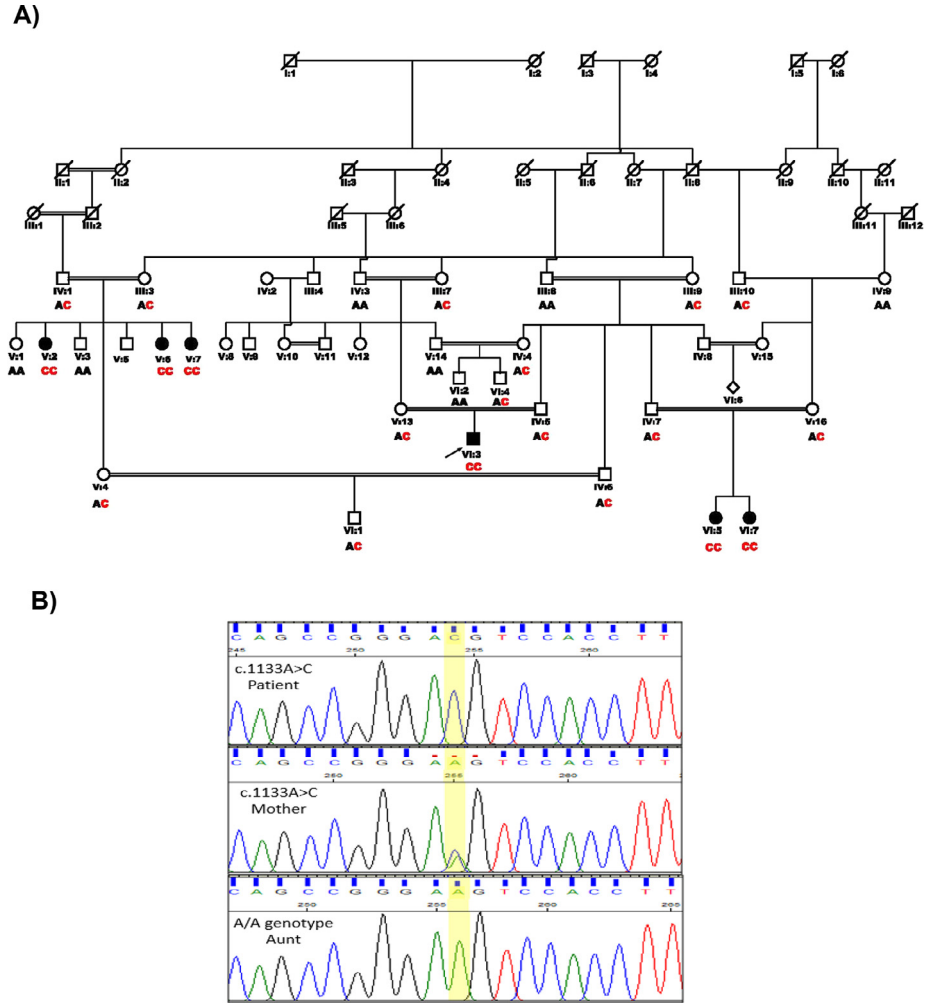

Fig. 1. A: The pedigree of the family. Genotypes of the variant c.1133A>C is shown in respected pedigree members. B: Chromatograms for the missense mutation in the proband, his heterozygous mother and his normal aunt, respectively.

Table 1
Clinical data of affected individuals.

Individual	Age (year)	Sex	Current head circumference (cm)	History of seizures
V:2	30	Female	NA	Yes (Tonic–clonic seizure)
V:6	27	Female	NA	No
V:7	18	Female	NA	No
VI:3	13	Male	47.5 (−4.5 SD)	Yes (age of onset: 4 year)
VI:5	5.8	Female	43.3 (−5.9 SD)	No
VI:7	3	Female	41.3 (−4.6 SD)	No

The variant was absent in all mentioned population databases, as well as in The Greater Middle East (GME) Variome (http://igm.ucsd.edu/gme/) and gnomAD browser (http://gnomad.broadinstitute.org/). This substitution was predicted to have damaging effect on the structure and function of the protein by SIFT (score = 0) and PolyPhen2 (score = 1.0) in silico tools, also it was predicted disease causing by Mutation Taster. The CADD-PHRED-score was 29. Bioinformatics tools support the evolutionary conservation of adenine nucleotide at position chr19:50365356, as well as, lysine residue at the position 378 of PNKP protein (Fig. 2a and b). (PhyloP and FastCons predictive tools; 3.5 and 1.0 respectively).

According to above analysis the c.1133A>C variant was selected for further validation in the pedigree. Sanger sequencing confirmed co-segregation of the variant with clinical features in 5 affected and 21 healthy family members. All non-affected parents were heterozygous (Fig. 1). Further, abnormal findings in nerve conduction

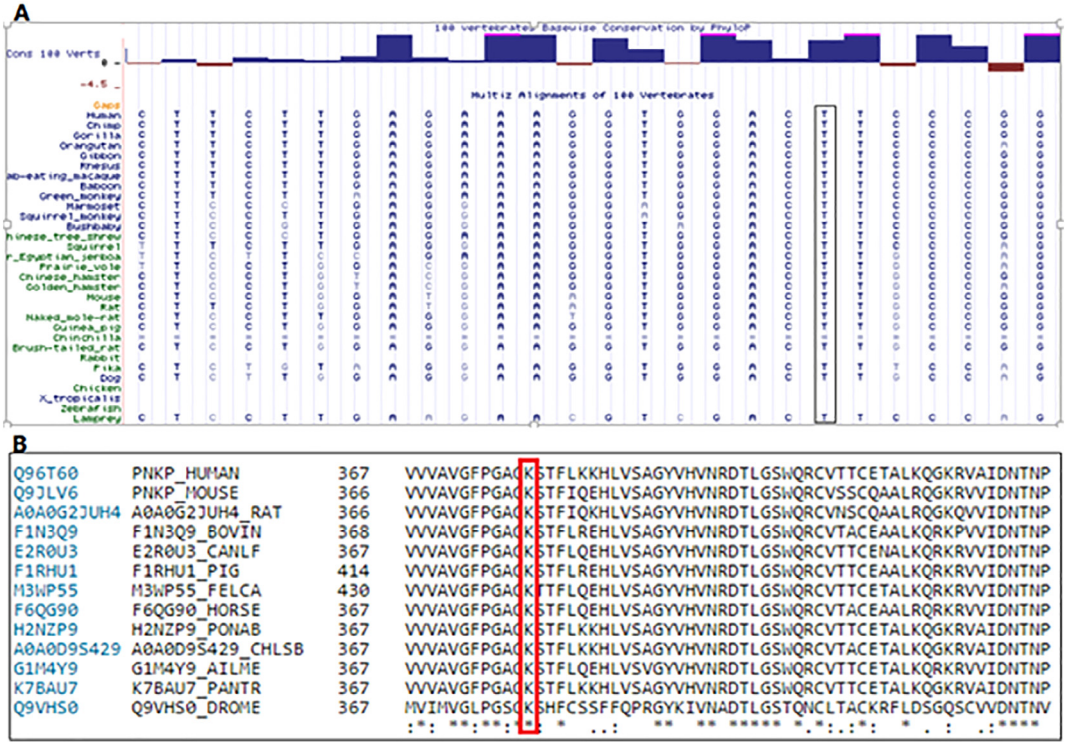

Fig. 2. Multispecies alignment for the candidate variant: c.1133A>C, p.Lys378Thr. (a) The panel from the UCSC genome browser (https://genome.ucsc.edu/cgi-bin/). (b) The panel from the UniProt (http://www.uniprot.org/align/).

velocity (NCV) confirmed the diagnosis of MCSZ syndrome in the family.

4. Discussion

Here, we reported a novel homozygous mutation c.1133A>C (p.Lys378Thr) in the *PNKP* gene related to phenotypes consisting microcephaly, seizure and developmental delay, in six patients from a large consanguineous Iranian pedigree. As the best of our knowledge there is no any previous report of this mutation in the literature.

The protein product of the *PNKP* gene, Polynucleotide Kinase 3′-Phosphatase, plays an important role in DNA repair pathways comprising the non-homologous end-joining (NHEJ) and base excision repair (BER) [7]. Mutations in this gene have been reported to be responsible in two distinct neurodevelopmental diseases comprising microcephaly, seizures, and developmental delay (MCSZ) and ataxia-oculomotor apraxia-4 (AOA4) [8].

Shen et al., identified different mutations of *PNKP* gene in MCSZ pedigrees, which most of them had a Middle Eastern origin [3]. Here we introduced a three-generation Iranian pedigree from the similar origin with the novel missense mutation in nearly the 3′ end of the protein. The protein contains three domains: the fork-head associated (FHA) domain in the N-terminal section which is responsible for recruiting the protein to the sites of DNA repair [9], and two DNA phosphatase and DNA kinase regions respectively from amino acid residues 146 to 337 and 341 to 516. The identified mutation located in the ATP binding site of the kinase domain (amino acids 372–379), in the highly conserved position. Previously reported mutations in this region were related to early-onset recessive ataxia with oculomotor apraxia (AOA), with no signs of microcephaly or epilepsy [8]. Thus, our findings support the previously suggested idea that either type or location of *PNKP* mutations do not seem to be related to the subsequent associated phenotypes. Analyzing all known causative genes in the category of neurodevelopmental disorders by traditional methods is infeasible. WES instead is an effective technology for determining the genetic cause of Mendelian disorders; even in complicated unsolved cases without defined clinical diagnosis [10]. Although targeted NGS panels are more accurate diagnostic tool in comparison to WES, in the case of indistinguishable clinical subgroups at the first glance as discussed here, WES seems more efficient. This study implies the application of WES in making the definite diagnosis in families with multiple affected individuals, through analyzing single patient. Furthermore, it can emphasis the role of genetic study as one of the preeminent lines in neurodevelopmental disorders.

Although multiple lines of evidences pointed out the pathogenic effect of c.1133A>C mutation in the PNKP gene, due to its novelty functional relevance of this mutation remains to be evaluated.

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