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ORIGINAL ARTICLE

A novel homozygous nonsense mutation in *CCDC88A* gene cause PEHO-like syndrome in consanguineous Saudi family

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Abstract

Progressive encephalopathy, edema, hypsarrhythmia, and optic atrophy (PEHO) syndrome is an unusual Mendelian phenotype of unidentified origin that causes profound intellectual disability, optic nerve/cerebellar atrophy, epileptic seizures, developmental progress, pedal edema, and early death. Uncharacteristic affected individuals are often classified as having PEHO-like syndrome, although they may be misdiagnosed as having epileptic encephalopathy, a potential result of early birth. In this study, we report a consanguineous Saudi family with a novel homozygous nonsense mutation of the *CCDC88A* gene causing PEHO-like syndrome. The children were suffering from developmental delay, epilepsy, mental disability, optic nerve/cerebellar atrophy, and pedal edema. Whole exome sequencing was conducted for the members of the family who have the disorder to study the novel mutation. Whole exome sequencing data analysis, confirmed by subsequent Sanger sequencing validation, identified a novel homozygous nonsense mutation c. 1292G > A, which was caused by p.Trp431* stop gain. This mutation was ruled out in 100 unrelated healthy controls. The nonsense homozygous mutation detected in this study has not yet been reported as pathogenic in the literature or various databases. In conclusion, a complete loss of protein function due to premature stop gain was caused by a mutation in exon 12 of CCDC88A. This loss may lead to PEHO phenotype. *CCDC88A* gene may therefore play an important and critical role for multiple aspects of normal human neurodevelopment.

Keywords *CCDC88A* gene · PEHO-like syndrome · Epilepsy · Intellectual disability · Saudi family

Introduction

Progressive encephalopathy is a neurological disorder that is present in general pediatric conditions. This disease usually found progressive encephalopathy with edema, hypsarrhythmia, and optic atrophy (PEHO). Children affected by PEHO syndrome may have undesirable psychomotor retardation and signs of progressive CNS diseases [1]. The prevalence of this disorder is unidentified but has been estimated to be 0.5–0.6 per 1000 live births [2]. Consanguineous marriage increases the risk of PEHO and other heredity disorders [2]. PEHO was described for the first time in Finnish children in 1991 [3]. In 1993, Somer et al. identified the important diagnostic criteria, which include the following: (1) hypotonia in the newborn baby; (2) seizures caused by epilepsy; (3) severe developmental delay, lack of motor signs, and delay in talking; (4) complete or partial damage in visual fixation and optic atrophy at an early stage; and (5) progressive atrophy of the cerebellum and pons [4]. Clinically, early infantile–epileptic encephalopathies have a phenotypic similarity with PEHO syndrome [5].

The affected patients in this study showed significant overlap with the CCDC88A-related PEHO-like syndrome regarding both microcephaly and early-onset epilepsy.

We found that variant mutation causes a premature stop gain within the *CCDC88A* protein at codon 431. Moreover,

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this truncating mutation is located upstream of the previously identified pathogenic mutation (p.Leu772X) [6]. In contrast to the expectation that a truncating mutation would lead to loss of function through nonsense-mediated decay, this mechanism seemed not to be activated upon introduction of the p.Leu772X mutation. Furthermore, the function of the truncated protein produced by this mutation was highly disrupted because of the lack of C-terminal domain [6]. Similarly, the premature stop at 431 will produce a truncated protein, which completely loses its function. In conclusion, detected homozygous mutation in the exon 12 of *CCDC88A* produces a complete functional loss in the protein, which leads to PEHO-like syndrome.

Method

Samples were collected from King Abdulaziz University Hospital, Jeddah, from the unaffected and affected family members. We followed the appropriate local ethical protocols and guidelines. Approval was obtained from each member who participated in this study. The investigation was also accepted by the ethical committee of the Center of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah. MRI and EEG were performed to exclude infection or trauma and to evaluate the severity and laterality of the disease. By using the MegNA Pure 24 system (Roche Life Science), DNA was extracted from patient blood samples. Detailed family pedigree was drawn after complete information form the family as shown in (Fig. 1). The samples were prepared for exome sequencing according to an Agilent Sure Select Target Enrichment Kit Preparation guide. Blood samples were taken from all four members of the family (two affected and two parents) and 100 unrelated unaffected people of Saudi origin as controls. Together, the patient and unaffected individuals underwent medical examination at King Abdulaziz University Hospital, Jeddah.

Patient 1 (IV-1) is 3 years old. The pregnancy period was normal and she was born via C-section delivery. She has microcephaly and mental retardation, and cannot walk or speak. She has delays in vision. The patient was diagnosed with epilepsy and usually has epileptic seizures lasting 30–40 s twice a day. The MRI examination showed dimorphic and brain atrophy. She has an affected brother with the same symptoms.

Patient 2 (IV-2) is 1-year-old boy with delays in vision. The MRI examination showed dysmorphic features and brain atrophy.

Whole exome sequencing

To identify the fundamental pathogenic change creating this disorder phenotype, we prepared whole exome using Illumina HiSeq 2000/2500. By following the Agilent SureSelect Target Enrichment Kit preparation guide (Capturekit, SureSelect_v6) we set up our sample. Those libraries were then sequenced using Illumina HiSeq 2000/2500. These variants were clarified using different parameters such as quality, frequency, genomic position, protein effect, and pathogenicity. Diverse bioinformatics investigations were conducted to distinguish causative variant co-segregating of *CCDC88A* phenotypes in an autosomal recessive manner. The crude information FASTQ files were adjusted using a BWA aligner (http://bio-bwa.Sourceforge.Net/). Insertion, deletion, and copy number variation were distinguished using SAMtools (http://samtools.Sourceforge.Net/). The resulting sequence reads were compared against the hg19 (NCBI manufacture GRCh37) human reference arrangement (http://genome.Ucsc.Edu/). The obtained data were then mapped against the information base in the Single Nucleotide Polymorphism database (dbSNP; (http://www.Ncbi.Nlm.Nih.Gov/snp/) and the 1000 Genomes database (http://www.1000genomes.Org/data). Separated variants were predicted based on autosomal recessive inheritance type (homozygous or compound heterozygous state) as reported in the family history. Because affected patients had similar histories, the homozygous variants were important. Our attempt to find novel homozygous transformations in the protein-coding districts of all genes that were related to one of the sings provided one reasonable candidate in the *CCDC88A* gene. Consequently, uncommon, possibly pathogenic variants found in this gene in the (compound) heterozygous (or

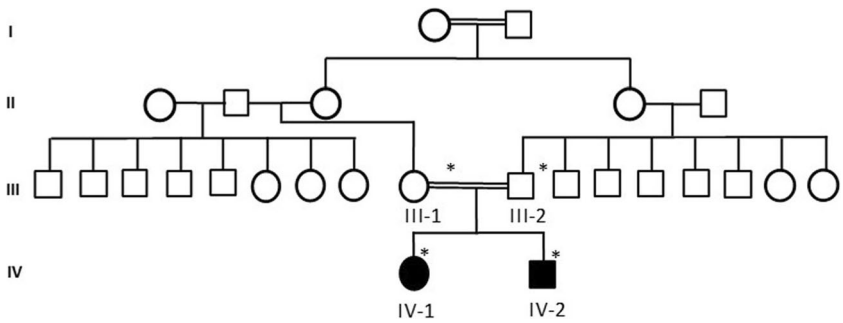

Fig. 1 Pedigree of a consanguineous Saudi family. The available samples are marked with an asterisk

Table 1 Mutation spectrum in *CCDC88A* gene known so far

S. No	Mutation	Consequence	State	Origin	Reference
1	c.5058 + 1G > A	changes the splice site donor G-U to A-U	Homozygous	–	Ekici et al. 2010
2	c.1715C > T	p.S572 L	Heterozygous	–	Ekici et al. 2010
3	c.5954C > G	p.S1985C	Heterozygous	–	Ekici et al. 2010
4	c.2313delT	p.Leu772*ter	Homozygous	Caucasian family	Nahorski et al. 2016
5	c. 1292G > A	p.Trp431* stop gained	Homozygous	Saudi Arabia	Present study

possibly de novo) state were considered. The investigation was then extended to all genes, whereby we initially investigated potentially destructive homozygous variations and then potential applicants in the (compound) heterozygous state. In any case, none of these methodologies yielded great hopeful variations, except for the novel homozygous nonsense mutation in *CCDC88A* gene.

Sanger sequencing

Exome sequencing data were further validated by using Sanger sequencing. Sanger sequencing was performed by designing the specific primer for mutation in the *CCDC88A* gene. The data obtained after sequencing using the BioEdit and FinchTV software were compared with the reference sequence. The detected results were examined in the National Center for Biotechnology Information (NCBI) SNP database. Additionally, we ran the same tests with 100 normal controls to confirm our results that the mutation is a pathogen.

Results

Whole exome sequencing

The variant call format file contains 86,390 variants. These results are filtered based on different criteria such as quality, genome position, frequency, the effect of the protein, and the phenotype. After filtering, homozygous variants in genes were considered. The results produced a homozygous nonsense mutation *CCDC88A* gene. A pathogenic mutation in the *CCDC88A* gene was discovered using whole exome sequencing. In exon 12, a novel mutation was found in G at position 1292 replaced by a resultantly amino acid glycine residue into a glutamic acid as a result in p.Trp431* stop gained in affected members of the family as shown in (Table 1). Additionally, in the greater Middle East, variome minor allele frequency was 0.00 in the database. Also, PolyPhen 0.05, SIFT 0.12, and mutation tester predicted this disease-causing mutation. This mutation was not found in the human gene mutation, OMIM, 1000 genome, or ExAc (http://exac.broadinstitute.org/) databases (Table 2).

Sanger sequencing

Our Sanger sequencing results showed a novel homozygous nonsense mutation, c. 1292G > A, which caused a stop gain in p.Trp431* in both the affected IV-1, and IV-2 probands, whereas the parents III-1 and III-2 were heterozygous at this position (Fig. 2). To validate this mutation as pathogenic, we sequenced 100 normal controls from the population and no one showed the same mutation.

Discussion

We detected homozygous *CCDC88A* mutation in the exon 12 of *CCDC88A* gene. This mutation caused a complete loss of protein function, which causes PEHO phenotype. The patients in this study has common clinical features including epileptic seizures at an early age, shortage in the developmental progress, different facial appearance, hypotonia, peripheral edema, and poor visual responsiveness [7, 8].

In the literature, we discovered 28 cases of PEHO from Finland [9], six cases from Japan [10], one from North

Table 2 Shows results of in silico tools used for prediction of pathogenicity

S. no	Online tools	Pathogenicity score
1	Diploid internal frequency	0.05
2	1000 genomes	0.0
3	Exome aggregation consortium	0.0
4	Polyphen	0.0
5	MutationTaster	1.0
6	MutationAssessor	0.0
7	PhyloP	0.94
8	Phastcons	1.0

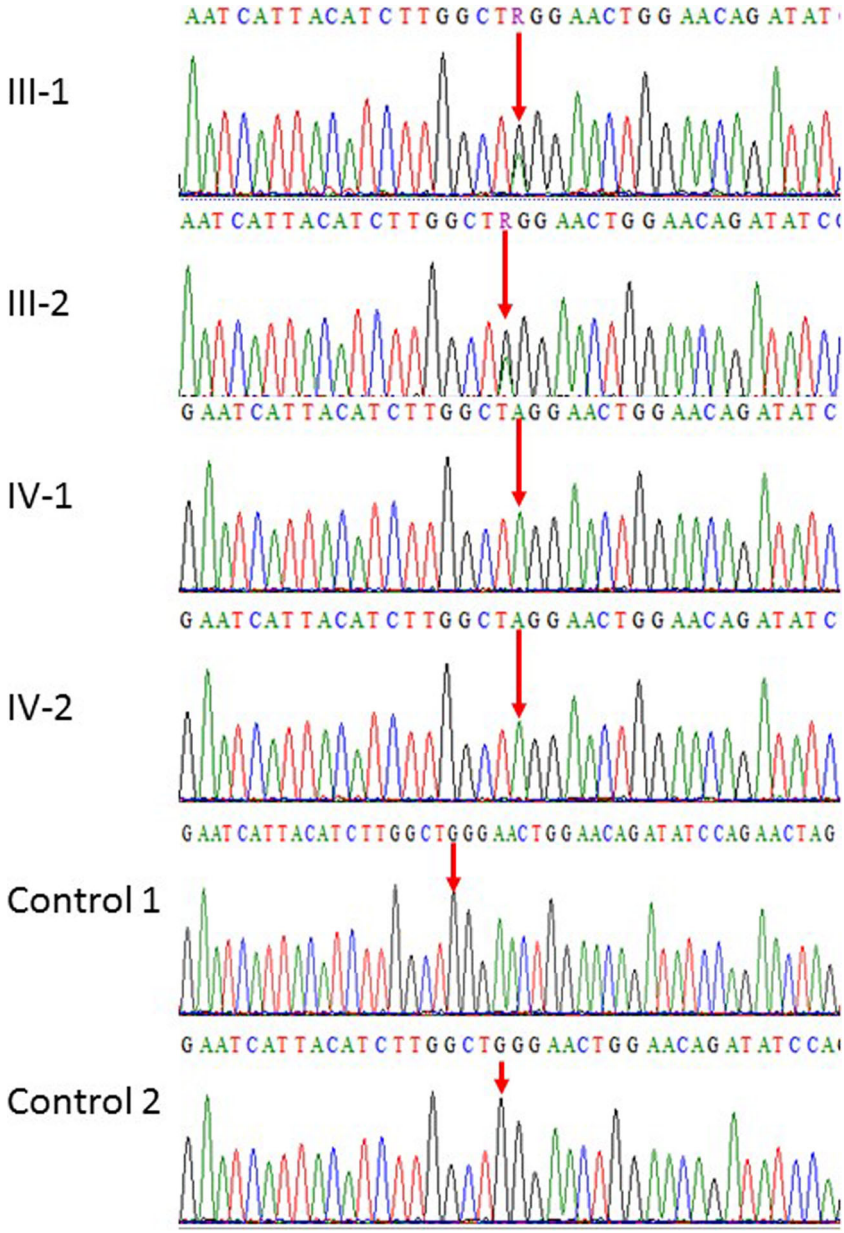

Fig. 2 Sanger sequence analysis: (III-1 and III-2) are normal parents showing G and A in heterozygous state, while (IV-1 and IV-2) are affected children showing only homozygous A in exon 12 of *CCDC88A* gene

America [3], and a report of four PEHO-like cases (including a pair of brothers) from Great Britain [7].

A novel mutation was identified in the *CCDC88A* gene. This gene is important in encoding a part of the Girdin family of coiled-coil domain–containing proteins. The resulting protein is an actin-binding protein that is stimulated by the serine/threonine kinase Akt and has a major function in remodeling the cell cytoskeleton and migration. Our protein also can affect Akt signaling by regulating phosphoinositide 3-kinase. Growth factor G protein–coupled receptors and growth factor receptor tyrosine kinases also stimulate Akt. If the expressions of this gene and the phosphorylation of the encoding protein increase, cancer metastasis results [11]. A single mutation has been detected in *CCDC88A*: a frame shift causing a premature stop codon (c.2313delT; p.Leu772X) has been recognized in three related patients from two families who presented with PEHO-like syndrome. Clinical diagnosis of these patients included congenital microcephaly, early-onset seizures (onset before 1 month of age), low muscle tone, motor mental slowing, progressive brain atrophy, and lack of visual fixation at an early age usually found with the optic disc degeneration [6].

In 2003, Longman et al. described two sisters with a PEHO-like disorder. The elder sister had epileptic seizures in early age, hypsarrhythmia, visual inattention with optic atrophy, progressive microcephaly, and lack of development [12].

Field et al. described five Australian patients, one of whom had classic characteristics of PEHO syndrome, while the other four had PEHO-like syndrome. The authors stated that the disorder might be more recurrent than would be suggested by the original diagnostic criteria [13].

Nahorski et al. recognized a homozygous 1-bp deletion (c.2313delT) in the *CCDC88A* gene. This mutation causes a frame shift and premature termination (L772X). It was found in three patients from one Caucasian family with PEHO-like syndrome [6]. Anntonen et al. reported a homozygous missense loss-of-function mutation c.92C4T (p.Ser31Leu) in a series of Finnish patients with PEHO syndrome [1]. Located in ZNHIT3 (MIM604500; NM_004773.3), this encodes the zinc finger HIT domain–containing protein 3.

In our study, we found a novel mutation in *CCDC88A* gene related to PEHO-like disorder caused by autosomal recessive pattern. We consider a *CCDC88A* gene to play a fundamental role in a normal, healthy human brain development.

Conclusions

PEHO disorder is a rare neurodegenerative syndrome that presents in childhood. The patient in our study shares many common features of PEHO disorder like the hypotonia, epileptic seizures, peripheral edema, characteristic dysmorphic features, and poor visual responsiveness. We report for the first time, a novel homozygous nonsense mutation in c.1292G > A in a Saudi family. This nonsense mutation alters protein p.Trp431* stop gain in *CCDC88A*, which may lead to PEHO-like disorders.

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Author's contributions M. I. N. conceived and designed the project. M. I. N. and A. A. A. performed experiments and confirmed these results. M. I. N., M. A. J., and A. G. C. analyzed and interpreted the data. M. I. N. and M. A. J. provided and interpreted phenotypic details for the patients. M. H. A., A. G. C., and K. O. A. advised on the study design and writing of the manuscript. M. I. N wrote the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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