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PEHO syndrome caused by compound heterozygote variants in *ZNHIT3* gene

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Abstract

PEHO syndrome is characterized by Progressive Encephalopathy with Edema, Hypsarrhythmia, and Optic atrophy, which was first described in Finnish patients. A homozygous missense substitution p.Ser31Leu in *ZNHIT3* was recently identified as the primary cause of PEHO syndrome in Finland. Variants in *ZNHIT3* have not been identified in patients with PEHO or PEHO-like syndrome in other populations. It has therefore been suggested that PEHO syndrome caused by *ZNHIT3* variants does not occur outside of the Finnish population. We describe the first patient outside Finland who carries compound heterozygous variants in *ZNHIT3* gene causing PEHO syndrome. Trio genome sequencing was carried out and the identified variants were confirmed by Sanger sequencing. The patient filled all diagnostic clinical criteria of PEHO syndrome. We identified biallelic missense variants in *ZNHIT3* gene: the c.92C>T p.(Ser31Leu) variant (NM_004773.3), which is described previously as causing PEHO syndrome and the second novel variant c.41G>T p.(Cys14Phe). There are only eight heterozygous carriers of c.41G>T

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variant in the gnomAD database and it is predicted damaging by multiple *in silico* algorithms. The *ZNHIT3*-associated PEHO syndrome exists outside of the Finnish population.

Keywords

PEHO syndrome; *ZNHIT3* gene; epileptic encephalopathy

Introduction

The PEHO syndrome (MIM 260565)-Progressive Encephalopathy with Edema, Hypsarrhythmia, and Optic atrophy, was first described by Salonen et al in 14 Finnish patients (Salonen et al., 1991). The diagnostic criteria include early onset seizures, infantile spasms with hypsarrhythmia, early arrest of mental development, profound hypotonia with no head support or ability to sit, and absent visual fixation from the first months of life (Somer 1993). Additional features include facial edema, microcephaly developing by 12 months of age, facial dysmorphism and optic atrophy. Brain MRI and CT show early progressive brain atrophy primarily involving the cerebellum and brainstem (Somer et al., 1993). Patients without optic nerve atrophy and brain imaging abnormalities, but fulfilling other PEHO criteria, are often described as a PEHO-like syndrome (Anttonen and Lehesjoki 2017). PEHO syndrome is most common in the Finnish population with an estimated incidence of 1:74,000 (Somer 1993). By combining homozygosity mapping in Finnish families with Sanger sequencing of positional candidate genes and with exome sequencing (ES) a homozygous missense substitution c.92C>T (p.Ser31Leu) in *ZNHIT3* was identified as the primary cause of PEHO syndrome (Anttonen et al., 2017). We describe the first patient outside of Finland who carries compound heterozygous variants in *ZNHIT3* gene causing PEHO syndrome; one is the recurrent Finnish variant, but the second is a novel variant.

Clinical report

The proband was born at term by Caesarean section due to breech position with birth weight 3190g (−0.5 SD), length 50 cm (0 SD), OFC 34.5 cm (−0.5 SD) and Apgar scores of 7 and 8. She is the only child in this family; family history is unremarkable.

At the age of one month, she was hospitalised due to muscular hypotonia and lethargy. Wolff-Parkinson-White syndrome was diagnosed on electrocardiography. Brain magnetic resonance imaging (MRI) are shown and described in Fig. 1A, C.

At the age of 6 months, her length was 67 cm (−1 SD), weight 7590g (−1 SD), OFC 41 cm (−1.5 SD). Facial phenotype is shown in Fig. 2A. Extremely severe muscular hypotonia, peculiar skin folds and profound developmental delay was noted. She had absent visual fixation, abnormal rotatory eye movements, and no head control. She had peculiar large nipples, but they were not inverted.

At the age of 10m generalized seizures started, first described as myoclonic, but later as infantile spasms. EEG showed hypsarrhythmia (multifocal burst suppressions from both

hemispheres) and antiepileptic treatment was administered. At the age of 1 year, she was hospitalized due to status epilepticus, which complicated by aspiration pneumonia, sepsis and a urinary tract infection. On clinical evaluation, microcephaly (OFC was 41 cm (-3.5 SD)) was once again noted and low weight 8.5 kg (-2.5 SD) was noted for the first time. In addition, she had severe central hypotonia with distal spasticity, exaggerated deep tendon reflexes, clonus, a positive Babinski sign, and feeding difficulties (requiring tube feeding). Brain MRI results are given in Fig. 1D and computed tomography (CT) scan results in Fig. 1B. At 17m of age, an ophthalmologist clarified that the child is blind: there was no pupillary reaction to light, absent eye fixation, chaotic movement of eye bulbus, pale and small disks, and temporal disk pallor (Fig. 2B,F).

At the age of 3.5 years she has progressive microcephaly (OFC 46.5 cm (-2.5 SD)), weight was 15 kg (-0.5 SD). She had no eye contact (Fig. 2C), tapering fingers (Fig. 2E), severe muscular hypotonia, dystonias, myoclonus, exaggerated deep tendon reflexes, and a positive Babinski sign and clonus bilaterally. EEG showed pathological slow background activity with multifocal and bilateral interictal epileptic activity. There were ictal episodes in sleep (myoclonus and two spasms). Brain MRI results are given in Fig. 1E.

At the age of 8.5 years, her height is 126 cm (-1 SD), weight 24.8 kg (-0.5 SD), OFC 47 cm (-4 SD). She has decerebrate posture with contractures, brisk tendon reflexes with clonuses, and scoliosis. Her face is edematous with an opened mouth appearance (Fig. 2D). She demonstrates the first signs of early puberty. She is fed by gastrostomy tube. The most recent EEG demonstrated extremely low background activity with multifocal bilateral interictal epileptic discharges and ictal tonic spasms on awakening. Brain MRI results are shown in Fig. 1F–G. Compared to the images at the age of 3y 6m there was minimal interval change.

She was tested for intrauterine infections. A metabolic workup, chromosomal microarray analysis, methylation test for Prader-Willi/Angelman syndrome, *COL4A1* and *JAM3* gene sequencing, muscle biopsy and an interferon level measurement in the serum and cerebrospinal fluid were performed with normal results.

The clinical features, EEG and brain MRI and CT results are summarized in Table 1.

Methods

Exome sequencing

Trio ES was initially performed in the clinical diagnostic setting at Tartu University Hospital (Estonia) to search for genetic variants in disease-associated genes. The exome was enriched using SureSelect Human All Exon V5 kit (Agilent), and sequenced on Illumina HiSeq platform.

Genome sequencing

Trio genome sequencing (GS) and data processing were performed by Genomics Platform at the Broad Institute of Harvard and MIT (Broad Institute, Cambridge, MA, USA). Sequencing libraries were prepared from 500 ng to 1.5 ug of genomic DNA using a PCR-

Free protocol. These libraries were sequenced on the Illumina HiSeq X Ten with 150 bp paired-end reads and a target mean coverage of >30×. Genome sequencing data was processed through a pipeline based on Picard, using base quality score recalibration and local realignment at known indels. We used the BWA-MEM aligner for mapping reads to the human genome build 37 (hg19). Single Nucleotide Polymorphism (SNPs) and insertions/deletions (indels) were jointly called across all samples using Genome Analysis Toolkit (GATK) HaplotypeCaller package version 3.4. Default filters were applied to SNP and indel calls using the GATK Variant Quality Score Recalibration (VQSR) approach. Lastly, the variants were annotated using Variant Effect Predictor (VEP) (McLaren et al., 2016). Written informed consent was obtained from the family for the routine clinical study and later for research.

RESULTS

Trio ES of the proband and parents did not identify clearly pathogenic variants in known genes causing Mendelian disorders. Analysis was performed one year before *ZNHIT3* was identified as a cause of PEHO syndrome.

On a trio-based GS analysis, variant filtration for *de novo* or recessive variants with allele frequencies below 1% in ExAC and gnomAD databases (Lek et al., 2016) revealed two high quality heterozygous missense variants in *ZNHIT3*. The c.92C>T p.(Ser31Leu) variant (NM_004773.3) was inherited from the mother and is described previously as causing PEHO syndrome in the homozygous state (Anttonen et al., 2017). The second variant c.41G>T p.(Cys14Phe) has not been reported as disease causing and was inherited from the father. There are only eight heterozygous carriers of the c.41G>T variant in gnomAD database and multiple *in silico* algorithms indicate a damaging effect (CADD 29, REVEL 0.9, PolyPhen2 0.999). This missense variant affects a highly conserved residue (conserved across species from *Drosophila* to human) in the HIT-type zinc finger (zf-HIT) domain. Both variants were confirmed on Sanger sequencing in the index patient and her parents (see Supplementary figure for the chromatograms of for both variants in this trio).

Reported variants have been submitted to the Global Variome shared Leiden Open Variation Database (patient ID: 00229643).

Discussion

We describe 8.5 years old female who fills all of the diagnostic clinical criteria of PEHO syndrome (Somer 1993) and carry compound heterozygosity for the “Finnish variant” and the very rare new variant, which affects a highly conserved residue in the zf-HIT domain.

After the initial description in 1991 (Salonen et al., 1991), the underlying etiology of PEHO syndrome remained unknown for many years. In 2017 Anttonen et al showed that loss-of-function of *ZNHIT3*, caused by homozygosity for the p.Ser31Leu variant, caused autosomal recessive PEHO syndrome in 26 Finnish PEHO patients (Anttonen et al., 2017). All of these patients fulfilled the diagnostic criteria for PEHO syndrome, except for infantile spasms (89%). Functional studies demonstrated that both knockdown and genome editing of *znhit3* in zebrafish embryos recapitulate the patients’ cerebellar defects, microcephaly and edema.

Moreover, knockdown of *Znhit3* in cultured mouse granule neurons and *ex vivo* cerebellar slices indicate that *ZNHIT3* is indispensable for granule neuron survival and migration (Anttonen et al., 2017).

During last few years, five other genes (*KIF1A*, *CCDC88A*, *GNAO1*, *CDKL5* and *PRUNE1*) has been described to cause PEHO or PEHO-like syndrome (Abdulkareem et al., 2019; Gawlinski et al., 2016; Langlois et al., 2016; Nahorski et al., 2016; Zollo et al., 2017). *De novo* disease-causing variants in *KIF1A* were first described as causing PEHO syndrome, but with large phenotypic variability even in patients with the same variants (Langlois et al., 2016). Soon after that, Nahorski et al described a consanguineous family of British origin with three children born with features of PEHO syndrome and a homozygous frameshift mutation in *CCDC88A* gene (Nahorski et al., 2016). Quite recently, another consanguineous family with a novel homozygous nonsense mutation of the *CCDC88A* gene causing PEHO-like syndrome was published (Abdulkareem et al., 2019). It has also recently been hypothesized that PEHO and PEHO-like syndrome may represent a severe end of the spectrum of the early-onset encephalopathies (Gawlinski et al., 2016). Among two patients with a prior diagnosis of PEHO syndrome and two patients with PEHO-like features, a disease-causing *de novo* variant in known genes (*GNAO1+HESX1* and *CDKL5*) was associated with neurodevelopmental disorders in two of four individuals. After the identification of *ZNHIT3* homozygous p.Ser31Leu variant in Finnish PEHO patients (Anttonen et al., 2017), an international group analysed a cohort of well-defined non-Finnish PEHO syndrome (n=9) and PEHO-like syndrome (n=15) cases from variable ancestries and failed to identify additional mutations in *ZNHIT3* (Salpietro et al., 2017). The same group recently characterized a new autosomal recessive neurodevelopmental and degenerative disorder caused by biallelic mutations in *PRUNE1*, with some of the affected individuals showing PEHO or PEHO-like features (Zollo et al., 2017). It was therefore concluded that autosomal recessive PEHO syndrome due to *ZNHIT3* variants is likely to be exceptionally rare outside of Finland, especially in association with the isolated founder p.Ser31Leu mutation. However, based on the available clinical data, these patients do not explicitly fulfill the diagnostic criteria of PEHO syndrome and thus are PEHO-like (Anttonen and Lehesjoki 2017).

Chitre et al recently evaluated 19 children from 11 families; seven had PEHO and 12 had PEHO-like syndrome and a pathogenic genetic variant was identified in 15 of them (*PCLO*, *SCN2A*, *PLAA*, *UBA5*, *CASK*, *CCDC88* and *SCN1A* gene (Chitre et al., 2018). Based on their results, the authors concluded that PEHO and PEHO-like syndrome are clinically and genetically diverse entities and represent the endpoint of many severe epileptic disorders.

It has been suggested that PEHO is no longer an appropriate clinical label outside of the Finnish population (Chitre et al., 2018). However, our described case argues against this point. Although Estonians and Finns are from the same Finno-Ugric language group, they are genetically rather different (Nelis et al., 2009). In our patient, the maternal p.Ser31Leu variant is classified as the Finnish PEHO allele, but as much as the mother knows of her family pedigree, she has no Finnish roots. The paternal p.Cys14Phe has not described before.

Conclusion

We describe the first case of the *ZNHIT3*-associated PEHO syndrome outside Finland. At the same time, we agree with the recent published data regarding the clinical and genetic heterogeneity of ‘PEHO spectrum disorders’ associated with variants in different progressive encephalopathy genes and the need for a comprehensive diagnostic work-up. We suggest that all the known genes causing PEHO and PEHO-like severe early-onset encephalopathies should be included in early-onset epileptic encephalopathy panels. In case of a severely ill child, the performing trio ES or GS is the most effective way for specifying the etiology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Abdulkareem AA, Abulnaja KO, Jan MM, Karim S, Rasool M et al. A novel homozygous nonsense mutation in *CCDC88A* gene cause PEHO-like syndrome in consanguineous Saudi family. 2019 *Neurol Sci* 40:299–303. [PubMed: 30392057]
- Anttonen AK, Laari A, Kousi M, Yang YJ, Jaaskelainen T et al. *ZNHIT3* is defective in PEHO syndrome, a severe encephalopathy with cerebellar granule neuron loss. 2017 *Brain* 140:1267–1279. [PubMed: 28335020]
- Anttonen AK, Lehesjoki AE. Reply: The phenotypic and molecular spectrum of PEHO syndrome and PEHO-like disorders. 2017 *Brain* 140:e50. [PubMed: 28899016]
- Chitre M, Nahorski MS, Stouffer K, Dunning-Davies B, Houston H et al. PEHO syndrome: the endpoint of different genetic epilepsies. 2018 *J Med Genet* 55:803–813. [PubMed: 30287594]
- Gawlinski P, Posmyk R, Gambin T, Sielicka D, Chorazy M et al. PEHO Syndrome May Represent Phenotypic Expansion at the Severe End of the Early-Onset Encephalopathies. 2016 *Pediatr Neurol* 60:83–87. [PubMed: 27343026]
- Langlois S, Tarailo-Graovac M, Sayson B, Drogemoller B, Swenerton A et al. De novo dominant variants affecting the motor domain of *KIF1A* are a cause of PEHO syndrome. 2016 *Eur J Hum Genet* 24:949–953. [PubMed: 26486474]
- Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E et al. Analysis of protein-coding genetic variation in 60,706 humans. 2016 *Nature* 536:285–291. [PubMed: 27535533]
- McLaren W, Gil L, Hunt SE, Riat HS, Ritchie GR et al. The Ensembl Variant Effect Predictor. 2016 *Genome Biol* 17:122. [PubMed: 27268795]
- Nahorski MS, Asai M, Wakeling E, Parker A, Asai N et al. *CCDC88A* mutations cause PEHO-like syndrome in humans and mouse. 2016 *Brain* 139:1036–1044. [PubMed: 26917597]
- Nelis M, Esko T, Magi R, Zimprich F, Zimprich A et al. Genetic structure of Europeans: a view from the North-East. 2009 *PLoS One* 4:e5472. [PubMed: 19424496]
- Salonen R, Somer M, Haltia M, Lorentz M, Norio R. Progressive encephalopathy with edema, hypsarrhythmia, and optic atrophy (PEHO syndrome). 1991 *Clin Genet* 39:287–293. [PubMed: 2070547]

- Salpietro V, Zollo M, Vandrovcsa J, Ryten M, Botia JA et al. The phenotypic and molecular spectrum of PEHO syndrome and PEHO-like disorders. 2017 *Brain* 140:e49. [PubMed: 28899015]
- Somer M Diagnostic criteria and genetics of the PEHO syndrome. 1993 *J Med Genet* 30:932–936. [PubMed: 8301648]
- Somer M, Salonen O, Pihko H, Norio R. PEHO syndrome (progressive encephalopathy with edema, hypsarrhythmia, and optic atrophy): neuroradiologic findings. 1993 *AJNR Am J Neuroradiol* 14:861–867. [PubMed: 8352158]
- Zollo M, Ahmed M, Ferrucci V, Salpietro V, Asadzadeh F et al. PRUNE is crucial for normal brain development and mutated in microcephaly with neurodevelopmental impairment. 2017 *Brain* 140:940–952. [PubMed: 28334956]

Figure 1:

At the age of 1 month T1 axial image (A) and T1 sagittal image (C) show mild cerebral atrophy with enlarged side ventricles and small occipital lobes with punctate high periventricular signal intensities (arrow) suspected for calcifications. At the age of 1 year CT (B) showed periventricular calcifications (arrow). At the age of 10 months T1 sagittal image (D) show progression of the cerebral, cerebellar and brainstem atrophy and thin corpus callosum. At the age of 3y and 6 months T2 axial image (E) show progression of the cerebral atrophy with small frontal and occipital lobes with loss of white matter and high signal intensities in periventricular region (arrow). At the age of 8 years 7 months T1 coronal image (F) shows severe cerebral and cerebellar atrophy (arrow), FLAIR axial image (G) shows almost no periventricular white matter with periventricular high signal intensities (black arrow). T1 sagittal images (H) shows thin and short corpus callosum (black arrow), severe cerebellar (white arrow) and brainstem (star) atrophy.

Figure 2:

(A) Facial view at the age of 6 months, note dolichocephalic shape of skull, abnormal eye fixation, epicanthic folds, wide nasal tip, and cupid bow mouth. (B) Facial view at the age of 10 months. (C) Facial view at the age of 3.5 years, no eye contact. (D) Facial view at the age of 3.5 years, note oedematous face with opened mouth appearance, no eye contact. (E) Tapering fingers and oedema of the hand. (F) Clearly bordered small disk, temporal disk pallor and diminished amount of peripheral blood vessels in retina.

Table 1:
Summary of the clinical features of index patient, EEG, and brain MRI and CT results.

	at birth	1 month	6 months	10 months	3.5 years	8.5 years
Height (cm, SD)	50 cm (0 SD)		67 cm (-1 SD)			126 cm (-1SD)
Weight (kg, SD)	3190g (-0.5 SD)		7590g (-1 SD)	8.5 kg (-2.5 SD)	15 kg (-0.5 SD)	24.8 kg (-0.5 SD)
OFC (cm, SD)	34.5 cm (-0.5 SD)		41 cm (-1.5 SD)	41 cm (-3.5 SD)	46.5 cm (-2.5 SD)	47 cm (-4 SD)
Facial features			Epicanthic folds, a wide nasal tip, and a Cupid's bow mouth (Fig. 2A)			Edematous with an opened mouth appearance (Fig. 2D)
Muscular tonus, reflexes	Normal	Muscular hypotonia and lethargy	Severe muscular hypotonia, no head control	Severe central hypotonia with distal spasticity, exaggerated deep tendon reflexes, clonus, a positive Babinski sign	Severe muscular hypotonia, dystonias, myoclonus, exaggerated deep tendon reflexes, and a positive Babinski sign and clonus bilaterally	Decerebrate posture with contractures, brisk tendon reflexes with clonuses
Development		Delayed	Profound DD	Profound DD	Profound DD	Profound DD
Seizures	Not noticed	Not noticed	Not noticed	Generalized seizures: myoclonias and infantile spasms	Generalized seizures	Generalized seizures
EEG				Hypsarrhythmia (multifocal burst suppressions from both hemispheres)	Pathological slow background activity with multifocal and bilateral interictal epileptic activity; ictal episodes in sleep (myoclonus and two spasms)	Extremely low background activity with multifocal bilateral interictal epileptic discharges and ictal tonic spasms on awakening
Vision			Absent visual fixation, abnormal rotatory eye movements	Blindness: there was no pupillary reaction to light, absent eye fixation, chaotic movement of eye bulbous, pale and small disks, and temporal disk pallor (Fig. 2B,F).	No eye contact (Fig. 2C)	No eye contact
Brain MRI		Mildly dilated lateral ventricles with porencephalic enlargement on the left and hypoplastic occipital lobes were seen with periventricular T1 hyper- and T2 hypointensities suspected to represent calcifications or blood (Fig. 1A). A thin corpus callosum and no myelin		Progressive cerebral atrophy, mostly in the occipital lobes, but also progressive brainstem and cerebellar atrophy (both vermis and hemispheres), and a thin corpus callosum (Fig. 1D).	Progressive brain atrophy, mostly in the occipital and frontal lobes with almost absent periventricular white matter with periventricular hyperintensities in T2 (Fig. 1E) and FLAIR sequences, progressive atrophy of the cerebellum and brainstem and preserved a thin and short corpus callosum with no interval growth from 10 months of age	Cerebral, cerebellar and brainstem atrophy, a thin and short corpus callosum and almost absent periventricular white matter with periventricular hyperintensities in T2 and FLAIR sequences (Fig. 1F-G). Compared to the age of 3y 6m there was minimal interval change

	at birth	1 month	6 months	10 months	3.5 years	8.5 years
Brain CT				Punctate calcifications in the periventricular white matter and in the thalamus on the right (Fig. 1B)		
Cardiological features		Wolff-Parkinson-White syndrome				Wolff-Parkinson-White syndrome
Feeding				Feeding difficulties (tube feeding)		Gastrostomy tube
Other symptoms			Dolichocephaly, closed anterior fontanelle, peculiar skin folds and large nipples		Tapering fingers (Fig. 2E)	Scoliosis; the first signs of early puberty