

CLP1 associated pontocerebellar hypoplasia

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Abstract

Pontocerebellar hypoplasia (PCH) is a group of hereditary neurodegenerative diseases characterized by the developmental pathology of infra tentorial brain structures such as cerebellum and pons. In recent years, many new PCH cases have been identified due to the evolution and easily accessible of genetic analysis such as next-generation sequencing.

We described a family were referred to our clinic because of epilepsy and MMR in two brothers with dysmorphic features: Physical examination revealed dysmorphic features such as; forehead sloping, hypertelorism, hypertrichosis, indistinct philtrum, cupped right ear, anteverted nares, oligodontia, high-arched palate, short neck, low posterior hairline, pes equinovarus, microcephaly (45cm, <1p (-4.7 SD)). Neurological examination showed evident dystonia in the extremities, increased deep tendon reflexes, spastic tetraparesis. Cranial MRI revealed pontocerebellar hypoplasia. Whole-exome sequence (WES) of two affected brothers revealed a homozygous p.R140H (c.G419A) (chr11:g.57,427,367 G > A [hg19], NM_006831.2) pathogenic variant in the *CLP1* gene which were described in 2014 in patients with similar clinical features.

In patients with pontocerebellar hypoplasia in MRI, if other causes in the differential diagnosis are ruled out except types and subtypes of PCH and MRI does not show dragon-fly like pattern *CLP1* gene can be sequenced first before whole exome or genome sequencing in patients with Asian origin which is much more cheaper and faster.

Keywords: Children; *CLP1*; pontocerebellar hypoplasia

INTRODUCTION

Pontocerebellar hypoplasia (PCH) is a group of hereditary neurodegenerative diseases characterized by the developmental pathology of infra tentorial brain structures such as cerebellum and pons. In recent years, many new PCH cases have been identified due to the evolution and easily accessible of genetic analysis such as next-generation sequencing (1). Cerebellar Hypoplasia (CH) has been classified according to affection parts into four groups such as; unilateral CH, CH with vermis involvement, global CH and PCH (1-4).

Specific major neurological findings of PCH with differences in subtypes are severe psychomotor retardation and speech delay. Dystonia / dyskinesia (chorea) or spasticity due to the alteration of cerebral cortex and subcortical nuclei are also common. Cortical structures usually unaffected, but generalized brain atrophy is common in prolonged lifespan. Clinical manifestations such as abnormal muscular tonicity, epileptic seizures and psychomotor retardation usually occur in early infantile period.

As mentioned before, one of the main primary causes of CH is the genetic disorders, so PCH classified at least into 10 types and several subtypes due to the affected gene (1,5). Pontocerebellar Hypoplasia, Type 10 (PCH10; OMIM # 615803) is associated to mutation in the homolog of the yeast cleavage and polyadenylation factor I subunit 1 gene (*CLP1*). *CLP1* gene located on chromosome 11q12.1 and encodes a multifunctional kinase protein which is a component of the tRNA splicing endonuclease complex and a component of the pre-mRNA cleavage complex II. This protein is plays role in tRNA, mRNA, and siRNA maturation (6).

In this work, we describe a Turkish family harbouring a *CLP1* gene mutation with differences in clinical manifestations in comparison with previously described PCH10 cases.

CASE REPORT

Family were referred to our clinic because of epilepsy and MMR in two brothers with dysmorphic features. First girl child of this consanguineous couples which had clinical similarities with brothers died at 9 months old. Cause of

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death is not clear but according to what family told about seizures and regular foaming at her mouth, aspiration is one of the possibilities.

Case 1; Eleven-year-old male patient, height 113 cm (<1p), weight 18 kg(<1p), head circumference 45cm (<1p) is a second child from consanguineous marriage (Figure 1). The prenatal history is unremarkable. He was admitted to hospital for the first time when he was 20 days old with tonic-clonic seizures. Head lifting occurred after 3 years old and he has never been able to sit and speak.

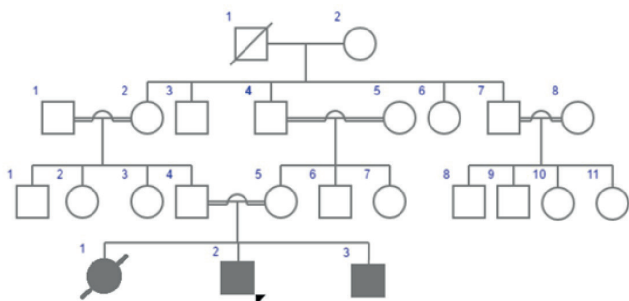


Figure 1. Consanguinity between normal parents supports autosomal recessive inheritance

Physical examination revealed dysmorphic features such as; forehead sloping, hypertelorism, anteverted nares, fullness of paranasal tissue, short and wide philtrum, diastema, high-arched palate, short neck, low posterior hairline, hypertrichosis (shoulders, back, arms, legs), pectus excavatum, right hand sydney line, pes equinovarus, bilateral overlap finger, bilateral arm and legs flexion contracture, microcephaly (45 cm, <1p (-6.2 SD)).

Neurological examination showed evident dystonia in the extremities, increased deep tendon reflexes, spastic tetraparesis. Routine laboratory (complete blood count, clinical chemistry) and metabolic tests (tandem mass spectrometry, blood, urine and cerebrospinal fluid amino acids and urine organic acid, ammonia, lactate, pyruvate) were normal. Hearing evaluation also was normal, eye exam showed mildly pale optic discs. Cranial magnetic resonance imaging (MRI) revealed pontocerebellar hypoplasia (Figure 2a). In electroencephalogram (EEG), severe widespread ground rhythm irregularity and slowing in frontocentral regions were observed. Electromyography (EMG) was suboptimal and motor unit potential duration motor and sensory nerve conduction velocities were normal.

Case 2; Six-year-old male patient, height 92 cm(<1p), weight 10 kg (<1p), head circumference 45 cm (<1p) is a third child from consanguineous marriage (Figure 1). He has a brother with the same clinical symptoms, same dysmorphic features and same physical-neurological examination.(previously described case 1). His prenatal history is also unremarkable as his brother.

Routine laboratory (complete blood count, clinical chemistry) and metabolic tests (tandem mass spectrometry, blood, urine and cerebrospinal fluid amino

acids and urine organic acid, ammonia, lactate, pyruvate) were normal. Hearing and eye evaluation also was normal. Cranial MRI revealed pontocerebellar hypoplasia (Figure 2b). Severe widespread ground rhythm irregularity was detected in the EEG. EMG showed demyelinating type neuropathy in the lower extremities, peroneal nerve conduction velocity was 33.6 m/s.

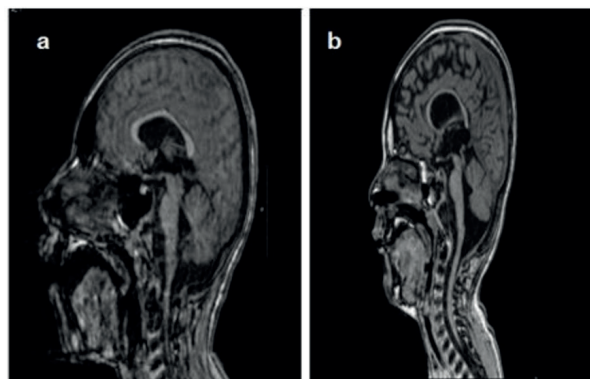


Figure 2. a,b. T1 weighted MRI of patients showing pontocerebellar hypoplasia.

Exome Sequencing was performed by using Ion Proton™ System (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's protocol. The Ion AmpliSeq™ Exome RDY Kit was used to construct DNA libraries. Samples were barcoded with Ion Xpress Barcode Adapters (Thermo Fisher Scientific, Waltham, MA). Library concentrations were quantified with Qubit® 2.0 Fluorometer before proceeding to emulsion PCR preparation. Following library measurement, Ion PI™ Hi Q™ OT2 200 Kit was used for amplifying library fragments with emulsion PCR on the Ion OneTouch™ 2 System. Exome Sequencing Analysis were performed for cases. Average base coverage depth was 78.1X and 93.88% of the mapped reads were aligned over a target region. The Torrent Mapping Alignment Program (TMAP) was used for aligning data to the hg19/GRCh37 human reference genome. We applied variant filtration steps to select and filter the variants detected in Exome Sequencing Analysis. Sanger sequencing analysis were performed for validation and segregation analysis.

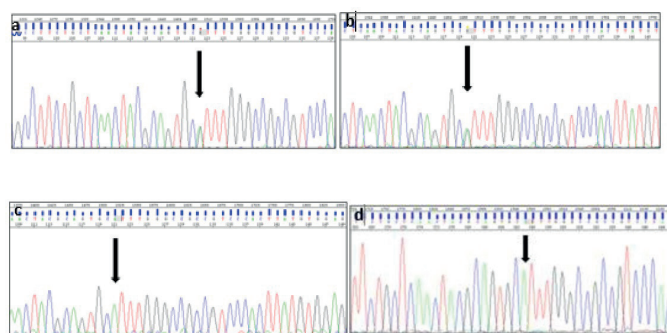


Figure 3. a) Mother (CLP1 gene c.419G>A(p.R140H) (heterozygous), b) Father (CLP1 gene c.419G>A(p.R140H) (heterozygous), c) Case 1 (CLP1 gene c.419G>A (p.R140H) (homozygous), d) Case 2 (CLP1 gene c.419G>A (p.R140H) (homozygous)

Whole-exome sequence (WES) of two affected brothers revealed a homozygous p.R140H (c.G419A) (chr11:g.57,427,367 G > A [hg19], NM_006831.2) pathogenic variant in the *CLP1* gene which were described in 2014 in patients with similar clinical features (Figure 3). Parents are revealed to be heterozygous carriers of this variant. Families described in study belong to the same ethnic group and reside in Eastern Turkey as our patients. Since *CLP1* gene alteration is associated with Pontocerebellar Hypoplasia, Type 10 (PCH10; OMIM # 615803) and clinical manifestations support this finding, the final diagnosis for our patients.

DISCUSSION

Neuropathologic and neurological findings assist the diagnosis of PCH but radiologic imaging is essential for diagnosis. There are also common dysmorphic features such as high arched eyebrows, prominent eyes, long palpebral fissures and eyelashes, broad nasal roots, hypoplastic alae nasi which were described in other cases in patients diagnosed with PCH which are not specific features assisting the diagnosis of PCH as described in our work, since none of these features were observed in our patients

Described MRI findings also can be seen in several other pathologies, so during differential diagnosis; carbohydrate-deficient glycoprotein syndrome type 1a and 1q were excluded due to the absence of abnormal fat distribution, coagulopathy, retinal degeneration, superimposed cerebellar atrophy, alpha dystroglycanopathies were excluded due to the normal vision and hearing and normal serum creatine kinase levels, PCH with cortical malformations were excluded due to the absence of cortical malformations in MRI, *CASK* gene related pathology were excluded because of its lethality in males (7).

The mutual Magnetic resonance imaging (MRI) findings of PCH are; hypoplastic cerebellum, the cerebellar hemispheres are reduced to bean-like or wing-like structures, markedly hypoplastic ventral pons, slight atrophy of the supratentorial gyral pattern, dilated cerebellomedullary cistern and fourth ventricle, delayed myelination of the white matter, no significant disorganisation of brain architecture and no severe corpus callosum defect (8). Cranial MRI revealed only pontocerebellar hypoplasia in our cases.

After exclusion bunch of possible causes there were still need in advanced investigation methods because of clinically undifferentiable pathologies such as Non-progressive cerebellar ataxias and PCH types and subtypes. So, genetic analysis by using whole exome sequencing method revealed homozygous pathogenic variant of *CLP1* gene which is responsible in PCH10 (6,9).

CLP1 gene product is multifunctional kinase protein takes part in tRNA, mRNA, and siRNA maturation. The pathogenic variants found by exome sequencing of this gene, segregated with the CH disorder in the families. Further

investigations revealed that with *CLP1* gene function loss there will be loss of kinase and cleavage activity and tRNA endonuclease complex function alteration, which will cause of an abnormal cellular accumulation unspliced pre-tRNAs. Schaffer et al. showed that *CLP1* null zebrafish will have cerebellar and motor degeneration and Hanada et al. revealed that homozygous *CLP1* mutant mice dies perinatally with spinal motor neuron degeneration and in human mutation of *CLP1* is considered as a responsible factor of PCH type 10 (6,9,10).

Case series describing PCH type 10 shows hypotonia, global growth retardation, truncal ataxia, abnormal eye movement, dysarthria, intentional tremor, microcephaly and seizures as a main clinical finding. Intellectual disability seen in more than 60% patients and 35% of which is severe, language speech disorders may range from moderate to total impairment and 5-20% of patients express autistic behaviour (6). Patients described in this work almost have all clinical characteristics mentioned above such as; microcephaly, global growth retardation, truncal ataxia, spasticity, epilepsy, severe intellectual disability, and total speech absence.

Namvar et al. described correlation between genetic analysis results and MRI findings in 169 radiologically diagnosed PCH patients from which 106 had TSEN or RARS2 mutation. No mutation was detected in 63 patients. TSEN mutation and MRI were found to be statistically significant between dragonfly like pattern. MRI did not show dragonfly pattern in our patients and which were revealed later they did not have TSEN or RARS2 mutation either (11).

Several articles about *CLP1* mutation related cases describe families that have no connection originates from Turkey and the fact that our patients' origin is close to this region suggests that this mutation might be related to the eastern Turkish origin (6,9,12).

CONCLUSION

In patients with pontocerebellar hypoplasia in MRI, if other causes in the differential diagnosis are ruled out except types and subtypes of PCH and MRI does not show dragonfly like pattern *CLP1* gene can be sequenced first before whole exome or genome sequencing in patients with Turkish origin which is more cheaper and faster.

Conflict of interest: The authors declare that they have no competing interest.

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