Understanding What You Have Found: A Family With a Mutation in the \textit{LAMA1} Gene With Literature Review

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\section*{ABSTRACT}

\textbf{INTRODUCTION:} Cerebellar dysplasia with cysts (CDC) is an imaging finding which is typically seen with individuals with dystroglycanopathy. One of the diseases causing this condition is “Poretti-Boltshauser Syndrome; PTBHS” (OMIM #615960). Homozygous or compound heterozygous mutations in the \textit{LAMA1} gene cause this disease.

\textbf{CASE PRESENTATION:} 7 years old twin siblings consulted to the medical genetics department because of walking problems and cerebellar examination findings.

\textbf{MANAGEMENT AND OUTCOME:} Clinical and radiological findings of the patient suggested a syndrome with recessive inheritance. Whole exome sequencing (WES) test was performed for definitive diagnosis. As a result of the patient’s WES analysis, a homozygous mutation was detected in the \textit{LAMA1} gene.

\textbf{DISCUSSION:} When determining the inheritance pattern of genetic diseases, if parents have consanguinity, this situation leads us to recessive inheritance diseases. Even if we are not consanguinity, but they say the same village, it is necessary to pay attention to the diseases of the recessive group. Whole exome sequencing analysis results in large amount of data generation. A good clinical evaluation is required to detect the mutation as a result of large data. To understand what we have found, we need to know what we are looking for.

\textbf{KEYWORDS:} Cerebellar vermis, cerebellar ataxia, laminin

\section*{Introduction}

Cerebellar malformations cause defective development of the cerebellum and often manifest itself in the first months of life. For some cerebellar malformations, neuroimaging findings are specific and provide diagnosis of disease.\textsuperscript{1} However, imaging findings of other cerebellar malformations such as hypoplasia, dysplasia, and cysts are less specific for the disease in which they are components.\textsuperscript{2,3} In 2014, “Poretti-Boltshauser Syndrome; PTBHS” (OMIM \#615960) is defined by cerebellar cyst and cerebellar dysplasia.\textsuperscript{4} This disease also causes myopia with or without retinal dystrophy.

Laminin is a multi-functional glycoprotein that is distributed at the interface between the basement membrane and extracellular matrix. It is composed of multiple subunits that are components of these heterotrimers.\textsuperscript{5} Of the 12 genes, 9 encoding the laminin subunits are associated with disease in human.\textsuperscript{6-9} One of these genes is the \textit{LAMA1} gene encoding Laminin alpha-1. The mutation in this gene causes congenital muscular dystrophy group diseases. In 2014, in the study by Aldinger et al., homozygous or compound heterozygous mutations in this gene were reported to cause “PTBHS.”\textsuperscript{10}

This disease is very rare. A total of 31 cases (we think 2 of them are repeated and same cases and therefore total number may be 29) of PTBHS have been reported in 4 publications.\textsuperscript{4,9-13} In this case report, we aimed to present the clinical findings of the siblings with homozygous mutations in the \textit{LAMA1} gene that caused PTBHS.

\section*{Case Presentation}

Twin boys aged 7 years consulted the medical genetics department with walking problem and cerebellar examination findings. Anamnesis information, examination findings, laboratory results, and genetic test results of twins are summarized in the following.

\textbf{Patient 1}

He was born at 38 weeks of gestation as 2300g. The 5-minute APGAR score was 8 to 9 points. He started walking at 2 years. At physical examination, head circumference, height, and weight were between 10 and 25 percentile. At the dysmorphic examination, narrow face, pointed chin, hypertelorism, telecanthus, macroxia, smooth philtrum, and thin upper lip vermilion were found. Hypokinesia and dysmetria were detected in the cerebellar examination of the patient. Also, he had ataxia when walking. At ophthalmic examination, he had normal fundus and he had no visual problems. The pathological findings at magnetic resonance imaging (MRI) of the patient are as follows: multiple cysts in millimetric dimensions at both cerebellar hemispheres, irregularities in the cerebellum cortex,
**Table 1. Summary of the literature of the cases of Poretti-Boltshauser Syndrome and our cases.**

| ALDINGE ET AL1 | P1 | P2 | P3 | P4 | P5 | P6 | P7 |
|----------------|----|----|----|----|----|----|----|
| Age            | 36 months | 36 months | 25 months | 29 years | 23 years | 5.5 years | 4.5 years |
| Sex            | Female | Female | Male | Female | Male | Female | Female |
| In LAMAT gene  | Homozygous | Compound heterozygous | Compound heterozygous | Compound heterozygous | Compound heterozygous | Compound heterozygous |
| Mutation       | c.588+2T>G | c.6345+3G>C (maternal) and deletion of exons 4-11 (paternal) | c.7965-15_7965-3del (maternal) and c.2988_2989delA (maternal) | c.6701delC (paternal) and c.8557-1G>C, c.768+1G>A (maternal) | c.6701delC (paternal) and c.8557-1G>C, c.768+1G>A (maternal) | c.2816_2817delAT (paternal) and c.5557>G (maternal) |
| Protein change | Splice and NA | Splice and p.Pro996Hisfs28* | p.Pro2334Leufs9* and splice, splice | p.Pro2334Leufs9* and p.Tyr185* | p.Tyr939Leufs27* and p.Tyr185* |
| Ethnicity      | Iranian | Mixed European | Mixed European | Mixed European | Mixed European | Asian and African American | Asian and African American |
| Occipitofrontal circumference | 50th percentile | >98th percentile | 20th percentile | 50th percentile | 30th percentile | 35th percentile | 35th percentile |
| Neurodevelopmental features | Moderate motor and speech delay | Moderate motor delay (no standing or walking), mild speech delay | Motor delay (cruising, but no walking), hypotonia | History of motor delay, normal speech, normal IQ, college graduate, lives independently | History of motor and speech delay, normal IQ, autism spectrum disorder (Asperger), lives with parents | Mild motor and speech delay, hypotonia | Motor and speech delay, hypotonia |
| Ataxia         | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown |
| Autistic features | − | − | − | − | − | − | − |
| Strabismus     | + | + | + | + | + | − | + |
| Brain MRI      | Cerebellar dysplasia | Cerebellar dysplasia | Cerebellar dysplasia | Cerebellar dysplasia | Cerebellar dysplasia | Cerebellar dysplasia | Cerebellar dysplasia |
|                | Vermis hypoplasia | Global | Global | Global | Global | Global | Inferior only |
|                | Superior cerebellar peduncles | Elevated and splayed | Elevated and splayed | Elevated and splayed | Elevated and splayed | Normal | Slightly elevated |
|                | Fourth ventricle | Enlarged | Enlarged | Enlarged | Enlarged | Mildly enlarged | Normal |
|                | Brainstem | Short pons, thin thalamus | Short pons, long midbrain, mildly enlarged tectum | Normal | Thin thalamus | Thin thalamus | Mass effect from arachnoid cyst |
|                | Ventricles | Normal | Moderate ventriculomegaly, partial agenesis of corpus callosum and septum pellicudum | Normal | Normal | Normal | Normal |
|                | Increased T2/FLAIR in white matter | Patchy increased, periventricular | Patchy increased, periventricular | Normal | Normal | Normal | Patchy increased, periventricular |
| Other          | Atrophic retina, aminoaciduria, consanguinity | Thinned retina, seizure | Absent pigment at retina | Lattice and peripheral degeneration at retina, macular heterotopia, increased pigment at retina, fatty liver on ultrasound, syndactyly in second and third toes | Atrophic retina, bilateral cataracts, echogenic liver on ultrasound, syndactyly in second and third toes | Retinal dysfunction: cones more affected than rods | Chorioretinal atrophy, macular and peripheral involvement, cones worse than rods |

Abbreviation: MRI, magnetic resonance imaging.
| VILBOUX ET AL. | MARLOW ET AL. | MASSON ET AL. | BANERJEE ET AL. | OUR CASES |
|--------------|--------------|--------------|----------------|----------|
| P1 | P2 | P3 | P1 | P2 | P1 | P1 | P2 |
| 21 years | 26 years | 8.5 years | Younger than 5 years | Younger than 5 years | 30 months | 2.5 years | 7 years | 7 years |
| Male | Female | Female | Female | Male | Female | Male | Male | Male |
| Compound heterozygous | Compound heterozygous | Compound heterozygous | Compound heterozygous | Compound heterozygous | Homozygous | Homozygous | Homozygous |
| c.6701delC (paternal) and c.678+1G→A, c.8557→C (maternal) | c.6701delC (paternal) and c.678+1G→A, c.8557→C (maternal) | c.2160T→A (maternal) and c.5985_5991del (paternal) | c.664C>T and c.2331C>G (maternal) | — | c.4702_4703del | c.6192C>A | c.6192C>A |
| Canonical splice and Pro2234Leufs*9 | Canonical splice and Pro2234Leufs*9 | p.Cys720* and p.Ile1966Glufs*7 | p.Arg222* and p.Tyr777* | p.Arg222* and p.Tyr777* | p.R2921* and exon 62-63 deletion | p.(Leu1568Glyfs*2) (p.S2731*) (p.S2731 *) (p.S2731 *) |
| Unknown | Unknown | Caucasian and Native American | Unknown | Unknown | Caucasian | Unknown | Turkish | Turkish |
| Normal | Normal | Unknown | Unknown | Unknown | Unknown | <3 percentile | 10-25 percentile | <3 percentile |
| History of motor and speech delay, graduated high school, mildly wide-based gait | Mild motor and speech delays, mildly wide-based gait, graduated from high school | Delayed motor development | Delayed | Delayed | Mild motor delay | Delayed | Mild motor delay, hypokinesia and dysmetria | Mild motor and speech delays |
| + | + | + | Unknown | Unknown | + | + | + | Unknown |
| − | − | − | Unknown | Unknown | + | Unknown | − | − |
| + | + | Unknown | Unknown | − | Unknown | + |
| + | + | + | + | + | + | + | + | The patient has no MRI |
| + | + | + | + | + | + | + | + |
| Normal | Normal | Normal | Atrophic | Atrophic | Normal | − | Normal |
| Enlarged | Enlarged | Normal | Enlarged | Enlarged | Enlarged | Enlarged | Enlarged |
| Midbrain is mildly elongated, pons and mildly reduced | Elongated midbrain and pons and appearing small | Normal | Normal | Normal | Unknown | Normal | Normal |
| Normal | Normal | Normal | Normal | Normal | Normal | Unkown | Normal |
| Normal | Normal | Normal | Normal | Normal | Normal | Bilateral periventricular white matter changes | Normal |
| Retinal dystrophy, high myopia, shoulder shrugging and nose wrinkling tics, bilateral syndactyly of the second and third toes | Shoulder shrugging and nose wrinkling, bilateral syndactyly of the second and third toes | Peripheral lattice degeneration of retina and bilateral arm extensions and flexions, nose wrinkling tics | High myopia | High myopia | Head tilt, occasional motor stereotypes, ocular motor apraxia | Myopia | Normal | Encephalocele, spasticity, pale optic disk |
cerebellar vermis hypoplasia, and enlargement at fourth ventricle (Figures 1 and 2).

**Patient 2**

In the prenatal period, encephalocele was detected in the seventh month of pregnancy. He was born at 38 weeks of gestation as 1900 g. The 5-minute APGAR score was 6 to 7 points. The encephalocele was excised at 16th day postnatal. The brain tissue and optic nerve were also located in this sac, so these structures were excised too. Hence, he is blind. He had ventriculoperitoneal shunt operation at the ninth month. When he was 5 years old, Botox was injected at the ankles because of spasticity in the lower extremities and tension in the Achilles tendon. He cannot walk still. At physical examination of the patient, his head circumference, height, and weight were under 3 percentile. He had microcephaly. His neck movement had limited extension. At the upper and lower extremities, he had spasticity. At the dysmorphic examination, narrow face, malar flattening, hypertelorism, upslanted palpebral fissure, telecanthus, macrotia, smooth philtrum, and thin upper lip vermilion were found. At ophthalmic examination, he had pale optic disk at the temporal regions in both eyes and he cannot see. At his cranial computed tomography, it was observed that lateral ventricles and third ventricular were collapsed.

The parents of these twin siblings have no consanguinity, but they are from the same village.

Whole exome sequencing (WES) was performed. As a result of this analysis, the homozygous NM_005559.4 c.8192C>A (p.Ser2731*) (p. Ser2731Ter) VCV000372974.1 in the \(LAMA1\) gene was detected (ClinVar ID is RCV000413473.1). This mutation is classified as disease causing in MutationTaster and the allele frequency is 0.0000159. In the analysis of the parents, the same mutation was resulted as heterozygous and these were again confirmed by Sanger sequencing. According to the American College of Medical Genetics (ACMG) criteria, this mutation has been reported as “pathogenic.” This is called “PTBHS” (OMIM #615960). Because of the genotype-phenotype similarity of the patients in the literature and our patients, we decided that the diagnosis was PTBHS. Thus, the definitive diagnosis was provided for twin brothers.

Additional informed consent was obtained from all individual participants for whom identifying information is included in this article. Ethical approval is not required at our institution to publish an anonymous case report.

**Discussion**

A total of 12 cases were reported in three publications for PTBHS (summarized in Table 1). Previously, in the study of Micalizzi et al., PTBHS patients from Turkey have been reported. We did not include Table 1 because they did not evaluate patients one by one in their articles. In addition, Table 2 summarizes the clinical and MRI findings of patients including the study of Micalizzi et al.

Previously, cases who are siblings have been reported, but identical twins cases have not been reported and encephalocele finding was also not previously reported in any cases. That may be wrong to think that \(LAMA1\) gene mutation may be the possible cause of encephalocele finding in P2. Because there is an
increased risk of congenital cranial malformations in twin cases.14,15 The compound heterozygous mutation is mostly observed. Previously, only one case reported had a homozygous mutation. At that case, consanguinity between parents was reported. Homozygous mutation was found in our cases too, but their parents had no consanguinity between. However, they were born in the same village. This situation suggests that they have consanguinity from distant ancestors. Therefore, it is very important to ask the patients where their parents are from. Because if they are from the same village, recessive inheritance disease should be considered.

At eye examination, most of the patients were found to have eye findings. At the eye examination, in one of our patients, any findings were found (P1). The possible cause of this condition can be considered as this mutation damage eyes less than other mutations. The probable cause of ocular finding in the other twin is encephalocele which develops during intrauterine period.

All mutations in a gene that cause a disease do not cause the same clinical findings due to mechanisms such as allelic heterogeneity and variable expressivity in genetic diseases. As shown in Table 1, the age at which PTBHS patients are diagnosed is on a wide range from 25 months to 29 years. The mean age of the patients presented in Table 1 (except our patient) was 10.10. In addition, Micalizzi et al. reported that the average age of follow-up of 17 patients with PTBHS was 7.2 years.9 MRI findings are present in almost all patients. However, eye examination findings and clinical findings such as ataxia and microcephaly were not reported in all the patients. This situation is an example for a genetic disease that causes a variety of clinical effects even in mutations in the same gene. As seen in our twin patients, although they have the same mutation but their severity for disease are different. Patients with mild clinical findings or with rare clinical findings in the disease may be challenging in diagnosis. In such a case, WES may be a preferable test.

Conclusions
The penetrance of all genetic diseases is different. This makes the diagnosis difficult. It is important to know the patient’s clinical history very well and to evaluate the patient in a multidisciplinary manner.

Acknowledgements
We thank our patient for agreeing to this report and sharing their insights on the genetic counselling experience.

Author Contributions
ME conceived and designed the study. BG performed clinical assessments. ME performed experiments and contributed to data acquisition, analysis, and interpretation. BG and MS drafted the manuscript. All authors contributed to critical revision of the manuscript for intellectual content and final approval of the manuscript.

Consent for Publication
Written informed consent was obtained from the patient’s parents for publication of this case report. A copy of the written consent is available for review by the Editor-in-Chief of this journal. The family and patient agree to publish this paper and have read and approved the final version of this manuscript including photos of the patient.

Ethical Approval
Ethical approval is not required at our institution to publish an anonymous case report.

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Table 2. Clinical and MRI features of cases reported as PTBHS (including our twin patients).

| FEATURES                  | NUMBER OF PATIENTS (%) |
|---------------------------|-------------------------|
| Sex (female)              | 16 of 33 (48)           |
| Ataxia                    | 22 of 33 (67)           |
| Strabismus                | 17 of 33 (52)           |
| Neurodevelopmental delay  | 32 of 33 (97)           |
| Myopia                    | 12 of 33 (36)           |
| Retinal dystrophy         | 15 of 33 (45)           |
| Cerebellar dysplasia      | 32 of 33 (97)           |
| Cerebellar cysts          | 31 of 33 (94)           |
| Enlarged fourth ventricle | 27 of 33 (82)           |
| Abnormal brainstem        | 15 of 33 (45)           |

Abbreviation: PTBHS, Poretti-Boltshauser Syndrome; MRI, magnetic resonance imaging.
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