The first Chinese Pierson syndrome with novel mutations in LAMB2

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Abstract

Background. Pierson syndrome is typically manifested with congenital nephrotic syndrome (CNS) and peculiar ocular changes. LAMB2 was the causative gene.

Methods. A 3.25-year-old girl presenting with childhood-onset heavy proteinuria, bilateral myosis and nystagmus was detected on mutations of LAMB2 gene by PCR direct sequencing.

Results. Two novel mutations were identified, C757fsX767 and P1413fsX1451, which predicted truncated proteins and were confirmed in the paternal and maternal origins, respectively.

Conclusions. This is the first Chinese case of Pierson syndrome diagnosed by clinical manifestations and LAMB2 gene mutations. The phenotype may be different in different ethics.

Keywords: autosomal recessive; genotype; myosis; Pierson syndrome

Introduction

Pierson syndrome (OMIM 609049) is an autosomal recessive disorder described by Pierson et al. in 1963 [1]. It typically comprises congenital nephrotic syndrome (CNS) and peculiar ocular maldevelopment [2]. Most cases started to show renal symptoms within the first 3 months of life, while some started during the gestational period with enlargement and hyperpigmentation of kidneys. The predominant renal histopathologic change was diffused mesangial sclerosis (DMS) [1–4]. Renal function quickly deteriorated, and most children would die during the neonatal period without renal replacement therapy. The peculiar ocular manifestation was extremely narrowing microcoria due to maldevelopment or atrophy of dilator. In 2004, Zenker et al. found that LAMB2 gene mutations were responsible for Pierson syndrome [2]. After that, 13 pedigrees of Caucasians and two pedigrees of Koreans were diagnosed and reported according to the clinical manifestations and molecular analysis [2–11]. It seemed that there were fewer cases in Orientals than in Caucasians. Are there any clinical and/or genetic characters in Oriental patients? And what is the relationship between the phenotype and genotype? When we had the first case of Pierson syndrome in the Chinese population, we analysed the clinical features, detected the LAMB2 mutation and compared our case with other ethical or regional patients previously reported by other authors. We hope the experience of any rare case could contribute to the whole spectrum of the disease in order to identify the disease clinically and to understand the disease pathogenetically.

Materials and methods

Patient

A 3.25-year-old girl was hospitalized with a complaint of large amount of foam in urine. Clinical characters were collected including urinalysis, renal function tests, blood analysis, renal biopsy examination and ophthalmologic examination. Family history was also investigated. Informed consent was obtained from the parents. Ethical committee approval was obtained from the Ethical Committee of Peking University First Hospital.

Molecular analysis

Genomic DNA was extracted from peripheral blood cells of the patient and her parents after obtaining informed consent. Sixteen pairs of primers were designed including all the 32 exons and exon-intron boundaries of LAMB2 gene through Primer 3.0 online. The PCR reaction was performed in a volume of 25 μl comprising of 12.5 μl 2× Taq plus Master Mix (Tiangen Biotech Co., Ltd., Beijing, China), 1 μl 5 pmol/μl sense primer and antisense primer, respectively, and 1 μl 50 ng/μl DNA. The amplification was carried out under ‘touchdown’ PCR procedure with the condition of annealing temperature from 64°C to 57 °C, descending 1 °C every 2 cycles, then annealing at 57 °C for 26 cycles. PCR products were visualized with 2% agarose gel electrophoresis and sequenced with ABI 3730XL (SinoGenoMax Company Limited, China). Meanwhile, we analysed the karyotype of the patient by amplifying the sex-determining region of Y chromosome (SRY). The sequences of sense primer and antisense primer were 5′-GAGTTGAAACCAGCCATGAC-3′ and 5′-TCTTTGAGGTGTTGGCTTT-3′, respectively. The reaction and amplification conditions were the same as those of LAMB2 gene. PCR products were visualized with 2% agarose gel electrophoresis. All PCR reactions were performed twice to make the results reliable. To confirm the abnormality of sequence analysis, a repeat sequencing was carried out from opposite direction.

Results

Laboratory tests revealed heavy proteinuria (3.07 g/24 h), microscopic haematuria, hypalbuminaemia (33.1 g/L), normal range 35–50 g/L) and normal renal function (BUN 3.2 mol/L, Ser 23 μmol/L) in this girl. The renal biopsy was performed at 3 years of age, which showed that 4/14 glomeruli had global sclerosis, the rest of the glomeruli showing mild and diffuse matrix extension, vacuolation of tubular epithelial cells and massive interstitial
inflammatory cells infiltration. Immunofluorescence staining on Ig and complements were all negative. Electron microscopy (EM) revealed mild proliferation of mesangial cells and extension of matrix, nearly normal structure of glomerular basement membrane (GBM) with a thickness of 161.86 ± 20.92 nm, and segmental effacement of podocyte processes (Figure 1). The girl presented with steroid resistance after 8 weeks' treatment. The ophthalmologic examination showed bilateral non-reactive microsia, myopia, nystagmus, cloudy vitreous bodies and remains of primary vitreous body. She had three patches of skin spots (milk coffee coloured) around her waist (0.7–1.5 cm²). A large placenta had been found on birth. Growth and development had been normal. The parents were not consanguinely married. The family history was unremarkable in addition to the microscopic haematuria (RBC 10–15/HP) detected in her mother.

The karyotype analysis revealed no Y chromosome. Two novel sequence variations in the open reading frame of LAMB2 gene were identified (Figure 2): a 73-bp deletion in exon 17 (c.2269–2341 del) predicting a frameshift mutation at position 757 and stop at 767 and a 1-bp deletion in exon 27 (c.4239 del A) leading to a frameshift mutation at position 1413 and stop at 1451. The father carried the same mutation as the girl in exon 17, and the mother carried the same mutation as the girl in exon 27.

**Discussion**

The female patient we reported here had heavy proteinuria (3.07 g/24 h), haematuria (BLD++) and distinct bilateral non-reactive microsia, which supported the diagnosis of Pierson syndrome. Further analysis of LAMB2 gene revealed novel compound heterozygous mutations, a 73-bp deletion in exon 17 (c.2269–2341 del) and a 1-bp deletion in exon 27 (c.4239 del A).

Pierson syndrome is a relatively rare autosomal recessive disorder constituted by CNS and peculiar ocular changes (bilateral non-reactive microsia) [1]. This syndrome is caused by GBM alterations resulting from LAMB2 gene mutations. LAMB2 gene analysis increased valid diagnosis of atypical Pierson syndrome, and recently variable phenotypes were reported. Atypical characters included late onset proteinuria, ocular abnormalities or nervous symptoms [8,10,11]. So far, there were only 15 families including 34 patients previously reported by others [2–11]. The onset ages of renal manifestations can be divided into three groups: within 3 months, 3 months to 1 year and after 1 year. The predominant onset age was within 3 months (13/15 families, 86.7%) [2,3,6–11]. Only in one family was the onset age between 3 months and 1 year (1/15 families, 6.7%) [8] and in one family was the onset age after 1 year (1/15 families, 6.7%) [7]. Twelve of 13 families with the onset age within 3 months underwent renal biopsies. Eight families presented with DMS [2,3,5,7,9,11], two families presented with focal segmental glomerular sclerosis (FSGS) [8,11], and two families presented with milt proliferation of the mesangium under the LM [7,10]. In 3/13 families in whom electron microscopic examination was performed, irregular contour of the GBM, thickening and layering of partial GBM and diffuse efface-
ment of the foot processes were revealed [5,10]. The patient with the onset age between 3 months and 1 year showed nearly normal under the LM, thinning and irregular contour of the GBM, fluffy material deposition on the subepithelial and subendothelial sides and widely effaced foot processes under the EM [8]. There were only two patients with onset age after 1 year, of which one presented with asymptomatic proteinuria and various increases of mesangial cells and matrix, another one with severe symptoms and glomerular sclerosis, abnormal GBM, diffused effacement of foot processes and tubular-interstitial changes [7]. The renal function in majority patients deteriorated within 1 year after onset. Eleven of 13 families with onset age within 3 months had renal failure within the first year of life and the other two at 1.3 years and 2.9 years, respectively [2,3,6–11]. The patients with onset age between 3 months and 1 year kept normal renal function up to 6 years [8]. In patients with onset age after 1 year, the renal function progressed differently from 1 year to normal renal function until 16 years of age. The girl we reported here had milder renal changes than previous cases and maintained normal renal function after 9 months of onset.

In the previous reports, 26/34 cases had ocular examinations. The typical ocular symptom was non-reactive microcyst as seen in 18/26 (69.2%) cases [2,3,5,8,9,11]. In addition, other abnormalities including nystagmus (4/26, 15.4%) [2,7,8], abnormal cornea (3/26, 11.5%) [2,3,9], abnormal lens (12/26, 46.2%), abnormal retina (8/26, 30.8%) [2,3,6,9,11], abnormal vision (5/26, 19.2%) [2,7,8,10,11] and remains of primary vitreous body (3/26, 11.5%) [2,5,11] were also reported. The girl we reported here presented with classic ocular abnormality and bilateral microcyst. Other symptoms were also previously reported, such as nervous symptoms, dyspnea, muscular hypotonia, growth and development retardation, psychomotor delay and atrial septal defect.

It is confirmed that Pierson syndrome is caused by mutations of LAMB2 gene, which is located at chromosome 3 [2]. The coding protein of LAMB2 is laminin beta 2 belonging to laminin family and has 1798 amino acids. Among all the cases with LAMB2 mutations, heterozygous compound mutations (10/15, 67%) were predominant [2,6,9,11]. Point mutations (14/28, 50%) and deletions (8/28, 28.6%) were the predominant nucleic changes. Most mutations resulted in truncated proteins (21/28, 75%) [2,6,8,9,11].

There were some undefined relations between phenotype and genotype. Firstly, missense mutations did not always exhibit mild phenotypes. In most cases with missense mutations, the renal manifestations were severe with very early disease-onset and fast deterioration of renal function [2,7,8]. Most missense mutations (3/4 families) localized at laminin N-terminal (LN) conserved domains and/or Domain I. These domains were critical for self-assembly of laminins or anchoring laminins to the GBM. Secondly, truncated mutations were not always related to severe phenotypes. There were two cases reported that had mild renal symptoms with bi-allelic truncated mutations. One is our case and the other was from Korea [8]. They had renal symptoms onset at 3 years and 8 months of age, respectively. They both had mild renal changes in LM and EM and kept normal renal function until 3 and 6 years old by follow-up, respectively. In addition, these two families are all Oriental, while others belong to Caucasians. Other patients [2,9,11] with bi-allelic truncated mutations all had severe renal symptoms. The relevant mechanism regarding to the mild renal symptoms with bi-allelic truncated mutations remained unknown. Thirdly, the same mutation may cause different phenotypes. In a family previously reported by others, there were seven patients identified with the same mutation but presenting with various phenotypes [6]. There must be some other factors affecting the phenotypes.

The girl’s mother did have microscopic haematuria (RBC 10–15/hp) and was detected with a single heterozygous LAMB2 mutation, which prompted that the carrier of LAMB2 mutation might present with renal symptoms. The mechanism needs further study.

In conclusion, the first case of Pierson syndrome from China was identified with novel compound heterozygous mutations on LAMB2, a 73-bp deletion in exon 17 (c.2269–2341 del) and a 1-bp deletion in exon 27 (c.4239 del A). There seemed no defined correlation between phenotype and genotype. The phenotype may be different in different ethics.

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Conflict of interest statement. None declared.

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