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RESEARCH ARTICLE

Detection of a new mutation (T1140C) in a patient with Hunter syndrome from Guangdong, China

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Abstract This study identified mutations of the idurnate-2-sulfatase (IDS) gene in a patient with Hunter syndrome, and established a basis for the diagnosis of the prenatal gene of Hunter syndrome. Urine glyeosaminoglycan (GAG) assay was used to make the preliminary diagnosis of mucopolysaccharidosis type II. Polymerase chain reaction (PCR) from dried blood spots and DNA sequencing were applied to analyze hotspot mutations in exons 9,3 and 8 of the IDS gene in the proband and his parents. A new missense mutation (T1140C) in exon 8 of the IDS gene was found by using DNA sequencing. This mutation caused a substitution of codon 339 from CTA (leucine) to CCA (praline). The patient is a hemizygote, and his mother is a heterozygote. The new missense mutation results in a change in the primary and tertiary structure of the IDS protein. It is possible that this mutation severely impairs enzymatic activity and is the underlying basis for the pathology seen in this patient with Hunter syndrome.

Keywords Hunter syndrome, mucopolysaccharidosis type II, iduronate-2-sulfatase, gene mutation, polymerase chain reaction, DNA sequencing

1 Introduction

Hunter syndrome is a severe and sometimes fatal X-linked recessive genetic disorder. Because of genetic mutations, a deficiency of lysosomal iduronate-2-sulfatase (IDS) function results in the accumulation of mucopolysaccharide in vivo and subsequently leads to higher excretion of dermatan sulfate (DS) and heparan sulfate (HS) in the urine. Clinically, a wide spectrum of phenotypes have been observed. The severe form is characterized by short stature, coarse facial features, mental and physical retardation, vascular and respiratory disorders. The patients with the severe form are usually presented with symptoms between two and four years old and come to a premature end with systemic dysfunction. In contrast, patients with the attenuated form could survive into adulthood, with little or no mental retardation. Hunter syndrome would progressively deteriorate with typical symptoms and have bad prognosis. At present, the treatment of this disease is uncertain. The commonly used way to detect the concentration of mucopolysaccharide in urine is only a preliminary diagnostic approach and assaying the activity of the enzyme also cannot define the gene carrier exactly. Therefore, both gene diagnosis and prenatal diagnosis are effective methods to prevent the disease (Zhang et al., 2004). We detect a new mutation (IDS) in a Chinese patient with Hunter syndrome from Guangdong Province and the results are shown as follows.

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2 Materials and methods

2.1 Materials

2.1.1 Patient

The patient, male, aged three and a half years, Han nationality, was investigated in this study. He was the first child of spontaneous labor at term. The patient was presented with progressive behavioral retardation, unstable walking and mental retardation after one year old, but he had normal hearing and eyesight, and could speak some simple words. He came to see a doctor in pediatrics in the First Affiliated Hospital, Sun Yat-sen University, and was sent to our laboratory to test for mucopolysaccharides (MPS) in the urine, which was suspected with the patient. The results show that DS(++), HS(++), CS(−), KS(−). Physical examination: height of 1.0 m, weight of 15 kg, claw hand, coarse skin, thickening hairs, olympic brow, short neck, big ears, tumbling bridge of the nose, lower hair line, abdominal distention, laparacele, unwieldy limbs and severe skeletal deformity. His father was 27 years old and his mother was 21 when he was born.

They have normal phenotypes and are not consanguineous marriage, either. Their families do not have similar case history. According to the clinical manifestation and the results of urine detection, it could be diagnosed as MPS II. After that, detection of IDS genetic mutation was done, and it was confirmed that he was a Hunter syndrome patient.

2.1.2 Primers and agents

Three primers were synthesized, and exons 9,3,8 of IDS were amplified according to the reference (Timms et al., 1998). Primer sequences were as follows: IDS9a: 5′-ATGTAACCCAT-TCTGCTCTG-3′,IDS9b:5′-GCTGGAA-GGGAGCACATC-3′; I2S3a: 5′-GCTGTTTGCTAGGAGC-CTCG-3′, IDS3b: 5′-CACTTTGGGTGAAAACGTGGC-3′; IDS8a: 5′-TCTGTGGTAATTCCAAGTG-3′, IDS8b: 5′-CCCCCAAAGCCTATGATTC-3′. These sequences were synthesized by BIOASIA Ltd, Shanghai. All agents were analytically pure which were made in China, besides dNTPs, Taq DNA polymerase (GENDA),100bp,123bp DNA ladder(GIBCO/BRL), SDS, proteolytic ferment K, agarose (Sigma).

2.2 Methods

2.2.1 DNA amplification by dried blood spot filter paper

One drop of peripheral blood was added to the filter paper, and dried in the open air. An eyehole was perforated, the diameter of which was about 3 mm, and put into an Eppendorf tube of 0.5 mL. It was added to 10 μL of freshly prepared methanol/acetone mixed liquor (with a volume score of one), and drawn out in 65°C vacuum. Each tube had PCR reaction liquid added as follows: each primer 20 pmol/L, 10*reaction buffer 5 μL, 2 mmol/L dNTP 5 μL, Taq DNA polymerase 2.5 U, twice-parboiled aquafer to make up to 50 μL. The PCR amplification about exons 9,3,8 of IDS was completed by DNA Thermal Cycler (PE corporation, USA) PCR instrument or Hema 480 type PCR amplification instrument (Hema Ltd, Zhuhai). The following PCR conditions were 95°C for 90 s, 35 cycles of 94°C for 40 s, 55°C for 30 s, and 72°C for 40 s, followed continued extension for 5 min, and then incubated for 4°C. 2.5 μL of the PCR product was taken from each sample and tested by 2% agarose minigel.

2.2.2 DNA sequence analysis

The PCR product was sent to BIOASIA Ltd., Shanghai directly to finish the two-way sequencing by ABI PRISM 3730 type or 377 type DNA sequence autoanalyzer (BIOASIA).

3 Results

According to the direct two-way sequencing about exon 8 of IDS in the patient, his mother and normal control, the results are shown in Figs. 1 and 2: the patient is a hemizygote of "T1140C" missense mutation, and his mother is a heterozygote of this mutation. Compared with the sequence using Blust software, it is known that the 339th codon inside the coding region of exon 8 (T is mutated into C of c DNA 1 140 bp) produced a new missense mutation. Figure 1 shows forward sequence in exon 8 of IDS about normal, his mother and the patient. The normal control sequence is 5′-GAACTAGGGTGGGCTCTAGGTGAACATGGAG-3′, his mother's heterozygosis sequence is 5′-GAACTAGGGT-GGGCTCC /T AGGTGAACATGGAG-3′. The mutational hemizygote sequence of the patient is 5′-GAACTAGGGT-GGGCTCCAGGTGAACATGGAG-3′. Single-line shows normal 5-methyl uracil (T), double-line shows heterozygotic basic group 2-hydroxy-6- aminopyrimidine/5-methyl uracil (C/T), and three-line shows mutational 2-hydroxy -6- amino-pyrimidine (C). Figure 2 shows reverse sequence in exon 8 of IDS about normal, his mother and the patient. Normal sequence is 5′-CTCCAT GTTCACCT AGAGCCCACCCTA GTT-3′, his mother's heterozygosis sequence is 5′-CTCCAT-GTTCACCTG/AGAGCCCACCC TA GTT-3′, the hemizygote sequence of the patient is 5′-CTCCATGTTCACCTGGAGCC CACCCTAGTT-3′. Single-line shows normal adenine (A), double-line shows heterozygotic basic group guanine/adenine (G/A), three-line shows mutational guanine (G).

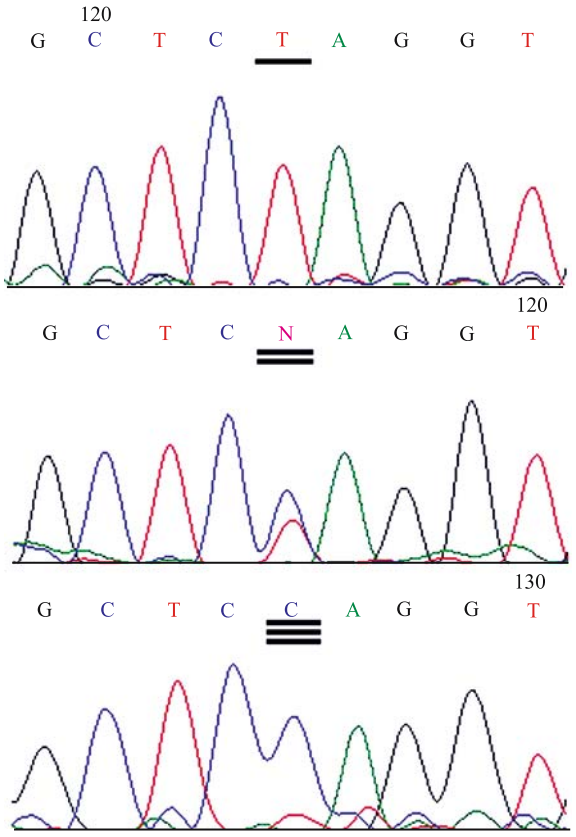

Fig. 1 They are the forward sequences in exon 8 of IDS gene of normal male (wild type), the patient's mother (heterozygote) and the patient (mutation hemizygote) from the top down

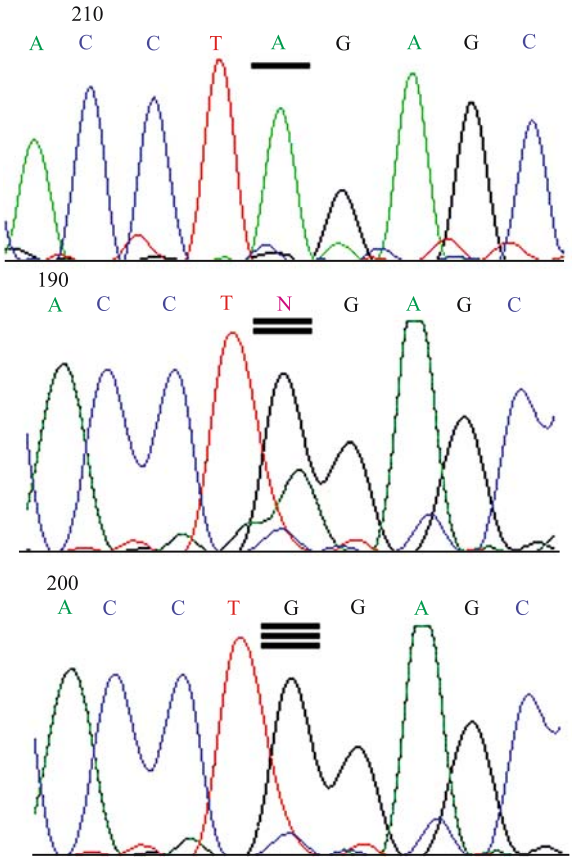

Fig. 2 They are the reverse sequences in exon 8 of IDS gene of normal male (wild type), the patient's mother (heterozygote) and the patient (mutation hemizygote) from the top down

4 Discussion

Hunter syndrome was first reported by a Canadian named Charles Hunter in 1917. It is a severe mutilational and fatal X-linked recessive genetic disorder. The incidence reported in the USA is 1:25 000 for live-birth male babies, while it is 1:132000 for male babies in China. Hunter syndrome is caused by a defect of lysosomal iduronate-2-sulfate, which induces degradation failure of DS and HS to deposit into organs and tissues. Its manifestations are evident across many major organs and tissues, including bones, joints, eyes, ears, teeth, skin, cardiac vessels, liver, spleen and the central nervous system. At the same time, it is accompanied with mucopolysaccharide urine, and has the characteristics of progressive deterioration (Timms et al., 1998).

The iduronate-2-sulfatase gene is located at the Xq27.32 ~Xq28. Three hundred and nine mutations of the IDS gene can be found using the GeneCards database from 1991 when the first mutation was reported by Wraith et al. (1991) to the time when this article was published. These genetic mutations can take place in every exon, especially in exon-9 (about 31%). The second is in exon-3, about 21% and the third is in exon-8, about 20% (Rathmann et al., 1996). The types of the mutation include missense mutation, nonsense mutation, shearing mutation, insertion mutation, deletion mutation, complicated rearrangement and so on (Chang et al., 2005; Chou et al., 2005; Guo et al., 2005; Guo et al., 2006; Li et al., 1999; Zhang et al., 2004). Molecular genetics research on Hunter syndrome was launched in foreign countries many years ago. For example, Machill et al. (1991) had studied and researched 158 patients with Hunter syndrome in 1991 and found that it had a high rate to detect a new mutation of the IDS gene (Hopwood et al., 1993). We analyzed the Chinese patient from Guangdong Province and found that this mutation is a new missense mutation which can not be found in the International Human Gene Mutation Database. We confirm that this mutation is not reported by literature retrieval.

Because this disease is an X-linked recessive inherited disease, the probability that another child born by the patient's mother is a patient or a carrier is 50%. Doctors diagnosed the disease only according to the change of the enzyme activity in peripheral blood before, so it was impossible to finally diagnose the intractable case, in which the patient was a carrier but had normal phenotypes without any symptom appearing. It is however important to screen carriers and diagnose pre-symptom patients at an early stage. It is not only good for making plans about occupation and marriage for those carriers and var. mitis or pre-symptom patients, but also good for breeding normal offspring by genetic counseling and prenatal diagnosis. We successfully screened exons 9,3,8 of IDS gene, and screen out T1140C mutation about exon 8. This mutation results in a missense mutation of codon 339 from CTA(leucine) to CCA(proline). It is a new missense mutation which has not been found yet according to the index. The findings are valuable for the elucidation of etiopathogenesis and the genealogical prenatal diagnosis or pregestational diagnosis for Hunter syndrome.

In addition, Carducci et al.'s (1992) upgraded method was used to directly amplify the specific DNA fragment from the dried blood filter paper. This method has the following five advantages. (1) Less blood is needed. Only a dried blood filter paper of 3 mm in diameter is needed. One drop of peripheral blood (about 0.04 mL) can be used for seven to ten tests. This method can not only alleviate the patients discomfort but also avoid the sample loss caused by the failure of abstracting the genomic DNA. (2) The outcome is sufficient and the specification is high. Therefore, the outcomes can directly be used for experiments such as DNA sequence analysis. (3) It is economical and feasible. Only some common biochemical reagents such as methyl alcohol and dimethylketone are needed, so it can be carried out in a general laboratory. (4) It is convenient to avoid the tedious process of abstracting the DNA in the procedure. (5) The samples are easily transported and preserved, so it is suitable for large scale sample collections in outlying areas. On account of so many advantages, this method is worth advertising and promoting.

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