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

GUO Yibin (✉), DU Chuanshu, WANG Jingjing
Department of Medical Genetics, Preclinical Medical School, Sun Yat-Sen University, Guangzhou 510080, China

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Abstract This study identified mutations of the iduronate-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 glycosaminoglycan (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.

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′-ATGTAACCAT-TCTGCTCTG-3′, IDS9b: 5′-GCTGGAAA-GGGAGCACATC-3′, I2S3a: 5′-GCTGTTTGCTAGGAGCCTCG-3′, IDS3b: 5′-CACCTTTGGTTGAAAACGTGGC-3′, IDS8a: 5′-TCTGTGGTAATTCCAAGTG-3′, IDS8b: 5′- CCCCCAACGCTATGATTC-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 cDNA 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′-GAACCTAGGGGTGGCCTTCAGGTGAACATGGAG-3′, his mother’s heterozygosis sequence is 5′-GAACCTAGGGGTGGCTCC/T AGGTGAACATGGAG-3′. The mutational hemizygote sequence of the patient is 5′-GAACCTAGGGGTGGCTCCAGGTGAACATGGAG-3′. Single-line shows normal 5-methyl uracil (T), double-line shows heterozygotic basic group 2-hydroxy-6- amino pyrimidine/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 AGAGCCCACCCA TA GTT-3′, his mother’s heterozygosis sequence is 5′-CTCCAT GTTCACCT AGAGCCCACCCA TA GTT-3′, the hemizygote sequence of the patient is 5′-CTCCAT GTTCACCT AGAGCCCACCCA TA GTT-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