Novel compound mutations of $SMARCAL1$ associated with severe Schimke immuno-osseous dysplasia in a Chinese patient

Zhihui Yue$^1$,*, Shiyi Xiong$^2$,*, Liangzhong Sun$^1$, Weijun Huang$^2$, Ying Mo$^1$, Liuyi Huang$^1$, Xiaoyun Jiang$^1$, Shumei Chen$^1$, Bin Hu$^2$ and Yiming Wang$^2$

$^1$Department of Paediatrics, The First Affiliated Hospital and $^2$Department of Medical Genetics, Center for Genome Research, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou 510080, PR China
Correspondence and offprint requests to: Liangzhong Sun; E-mail: sunlzh@mail.sysu.edu.cn
*These two authors contributed equally to this work.

Abstract

Background. Schimke immuno-osseous dysplasia (SIOD) is a rare autosomal recessive pleiotropic disease caused by mutations in the $SMARCAL1$ gene. To date, there have been no data from the Chinese population. Here, we report the first SIOD case in the Chinese population. No case with gross carpal bone age retardation has been reported previously.

Methods. The index patient was diagnosed by clinical and laboratory investigations. Mutations analysis of the $SMARCAL1$ gene and haplotype analysis were performed in the family. Structural predictions of the wild-type and mutant proteins were conducted.

Results. Severe SIOD was diagnosed in an 8-year-old boy, who exhibited growth failure, recurrent infection, neutropaenia, spondyloepiphyseal dysplasia, focal segmental glomerulosclerosis, T cell immunodeficiency and facial dysmorphism. Marked carpal bone age retardation was also observed. Sequence analysis of the $SMARCAL1$ gene revealed two novel mutations: c.3G>A (p.Met1?) and c.1682G>A (p.Arg561His) in the boy. Haplotype analysis and mutation detection showed that the father is the carrier of c.3G>A (p.Met1?) and the mother is the carrier of c.1682G>A (p.Arg561His). The paternal mutation, c.3G>A (p.Met1?), is predicted to introduce a new open reading frame, resulting in truncation of 103 amino acids at the N-terminus. The maternal mutation occurs in the SNF2-related domain involved in ATP hydrolyzation and DNA binding and is predicted to alter the local spatial structure of the protein.

Conclusion. We report the first SIOD patient from China, who exhibited gross carpal bone age retardation and carried two novel mutations, c.3G>A (p.Met1?) and c.1682G>A (p.Arg561His), in the $SMARCAL1$ gene.

Keywords: bone age retardation; focal segmental glomerulosclerosis; Schimke immuno-osseous dysplasia; $SMARCAL1$; spondyloepiphyseal dysplasia

Introduction

Schimke immuno-osseous dysplasia (SIOD) (Online Mendelian Inheritance in Man (OMIM) # 242900) is a rare autosomal recessive inherited disease first reported by Schimke et al. in 1971 [1]. It is characterized by growth failure from spondyloepiphyseal dysplasia, recurrent infections from cellular immune defects, progressive renal failure with nephrotic range of proteinuria and facial dysmorphism [2,3]. Some patients also suffer from hypothyroidism, episodic cerebral ischaemia and bone marrow failure [3]. According to the severity of clinical manifestations, SIOD can be classified into two different subtypes: severe and benign variants (mild form) [2,4]. Patients with the severe form suffer from intrauterine growth retardation, severe growth failure after birth, recurrent infections, haematological abnormalities, hypothyroidism, cerebrovascular disease and often die within the first 15 years, mostly in the first 2 to 5 years of life from severe infection, renal failure or cerebrovascular disease. Patients with the mild form usually present with growth failure and renal dysfunction between 8 and 13 years and progress to renal failure over the next 6 to 12 years and do not suffer from infections or cerebrovascular disease [2,3].

The SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a-like 1 gene ($SMARCAL1$; Entrez gene ID: 50485) encodes human hepA-related protein (HHARP) [5]. HHARP is known to function as an ATP-driven annealing helicase [6], and $SMARCAL1$ is believed to be the gene underlying SIOD. Its mutations are reported from several populations [3,7]. However, there have been no data from the Chinese populations. Grossly retarded bone age has not previously been reported. In this paper, we report clinical and molecular findings of the first SIOD case identified in Chinese populations.
Materials and methods

Patient, his family and control subjects

The study was carried out in accordance with the principles outlined in the Declaration of Helsinki. It was approved by the ethics committee of Sun Yat-sen University. Informed consent, approved by the First Affiliated Hospital of Sun Yat-sen University, was obtained from all subjects.

The index patient was an 8-year-old boy from a healthy non-consanguineous couple of Chinese Han ethnicity from Fujian province. Routine clinical and laboratory examinations, including haematological and immunological tests and skeletal X-ray, were performed on the whole family. The boy also underwent further examination with renal biopsy, growth hormone stimulating test and thyroid function tests. Both the patient and his parents were diagnosed by two independent paediatricians. All diagnoses were made according to stated ascertainment [1,2,4,8]. The disease severity of this patient was scored according to the criterion suggested by Clewng et al. [7]. Severe patient was diagnosed when the total score was 4 or more points.

Fifty healthy individuals without signs or symptoms of SIOD were recruited from the medical examination centre of our hospital.

Mutation detection of SMARCAL1 gene and haplotype analysis

DNA was extracted with the QIaamp DNA Blood Maxi Kits (Qiagen, Hilden, Germany). Primers for coding, intron–exon boundaries with extensions into intronic regions from 127 to 570 bp and the 5′ and 3′ flanking regions of the SMARCAL1 gene (Entrez gene ID: 504858) were designed by Primer Premier 5. (The primer sequences and PCR conditions are available upon request). The PCR products were sequenced in both directions on the ABI Genetic Analyzer 3730XL (Applied Biosystems, Foster City, CA), with a BigDye Terminator v3.1 Cycle Sequencing Kit, and results were compared with sequences retrieved from the University of California at Santa Cruz (UCSC) Genome Browser (http://genome.ucsc.edu). Haplotypes were constructed by Cyrillic 2.1 (http://www.cyllicsoftware.com/) with markers spanning the locus (D2S2361, D2S126, Applied Biosystems, Foster City, CA) and mutations identified in the family. The mutation nomenclature used here is in accordance with guidelines for describing sequence variations (www.hgvs.org/mutnomen/), with +1 corresponding to the A of the ATG translation initiation codon of the GenBank cDNA sequence NM_014140.3 and the amino acid sequences NP_504859.2.

Bioinformatics analysis

CLUSTAL X (1.83) [9] was used to compare the human SMARCAL1 amino acid sequences (Homo sapiens, NP_504859.2) with five orthologues: chimpanzee (Pan troglodytes NP_001155124.1), dog (Canis familiaris, XP_536062.2), rat (Rattus norvegicus, NP_00101692.1), mouse (Mus musculus NP_061287.1) and nematode (Caenorhabditis elegans, NP_498400.1). The NCBI ORF Finder (http://www.ncbi.nlm.nih.gov/ gor/gorf.html) was used to identify changes in the open reading frame (ORF) for the sequences NM_014140.3 with the c.3G>A mutation. The topological structures of wild-type and mutant proteins were modelled by means of the PSSPRED Protein Structure Prediction Server (http://bioinf.cs.ucd.ac.uk/psspred/pssform.html).

Results

Clinical characteristics

The index patient was from Fujian province, China. He was the first and only child of non-consanguineous, Chinese descent parents (mother, 29 years old; father, 30 years old). Both parents have normal stature. The father was healthy but the mother had hyperthyroidism. The boy was born at 37 weeks of gestation and spontaneously delivered with a weight of 1.9 kg and a length of 40 cm, which were all below the third centile.

The patient was referred to our hospital at the age of 8 years and 2 months. He suffered from severe growth retardation since gestation and grew poorly postnatally. Since 2 years and 8 months of age, the boy had experienced recurrent infections (pneumonia and diarrhoea), intermittent lymphopenia, neutropenia and anaemia. He exhibited massive proteinuria since 6 years and 3 months of age and oedema in the lower limbs since 8 years and 2 months. He had normal intelligence. Physical examination on admission demonstrated disproportionate short stature (83.5 cm in height and 11.5 kg in weight with a short neck and trunk) with a protruding abdomen and hyperpigmented macules on the face and trunk. He had a triangular face, broad, low nasal bridge and bulbous nasal tip (Figure 1A–C).

Skeletal X-ray presented carpal bone age of 4 years [10] with four ossification centres (Figure 1D), spondyloepiphyseal dysplasia with ovoid, flat vertebrate and osteopenia (Figure 1E). Renal features included nephrotic range of proteinuria (100 mg/kg d) (normal ranges, 50–80 mg/24 h) [11], hypoaalbuminaemia (18 g/l) (normal ranges, 35–50 g/l) [11], oedema and defect renal function (clearance of creatine, 54 ml/min 1.73 m²) (normal ranges, 97–137 ml/min 1.73 m²) [11]. A renal biopsy showed focal segmental glomerulosclerosis (Figure 1F–G). Haematological and immunological studies showed hypoproteinaemia (0.34 × 10³/l) (normal ranges, 1.5–3 × 10³/l) [11], neutropenia (0.2 × 10³/l–1.73 × 10³/l) (normal ranges, 3–5.8 × 10³/l) [11] and anaemia (haemoglobin 70 g/l) (normal ranges, 115–155 g/l) [11]. T cell immunodeficiency included low levels of CD³⁺, CD⁴⁺ and CD⁸⁺ T cells (9.1, 2.7 and 6.4%, respectively) (normal ranges, CD³⁺ T cells 66–76%; CD⁴⁺ T cells 33–41%; CD⁸⁺ T cells 27–35%) [12] and a low ratio of CD⁴⁺/CD⁸⁺ (0.42) (normal range, 1.1–1.4:1) [12]. Thyroid function was normal. Results of growth hormone stimulating test with arginine were normal: 2.9 ng/ml (empty stomach), 10.4 ng/ml (30 min after arginine intravenous injection), 6.28 ng/ml (after 60 min), 6.09 ng/ml (after 90 min) and 3.86 ng/ml (after 120 min). (Peak level of growth hormone <7 ng/ml is compatible with growth hormone deficiency [11].)

A 7-week steroid therapy trial and angiotensin-converting enzyme inhibitor enalapril were ineffective and the patient died of severe infection (diarrhoea) at 8 years and 4 months.

According to the criteria suggested by Clewng et al. [7], a total score of 6 points was obtained and severe SIOD was diagnosed (Table 1).

Both parents were normal on physical examination, urinalysis, granulocyte count, total lymphocyte count and number of CD3⁺, CD⁴⁺ and CD⁸⁺ T cells. Lateral spine radiographs of the parents were also normal.

Mutation and haplotype analysis

Direct sequencing of both DNA strands of the SMARCAL1 gene in the patient revealed two novel mutations: c.3G>A (p.Met1?) and c.1682G>A (p.Arg561His). The patient was a compound heterozygous of these two mutations. Haplotype analysis and sequencing showed that the father was a carrier of the c.3G>A (p.Met1?) mutation and the mother was a carrier of the c.1682G>A (p.Arg561His) mutation (Figure 2A–E). Sequencing of
SMARCAL1 in 100 control chromosomes did not identify the c.3G>A and c.1682G>A mutations in any individuals, suggesting that the c.3G>A and c.1682G>A substitutions were not polymorphisms.

Bioinformatic analysis

Alignment of the SMARCAL1 gene across six different species showed conserved methionine and arginine at the
1st and 561st amino acid in human, chimpanzee, dog, rat, mouse and nematode (Figure 3A and B).

The paternal mutation, c.3G>A (p.Met1?), occurred in the translation initiation codon ATG. This is predicted to introduce an alternative ORF from a second ATG 310 bp downstream of the normal translation initiation codon, leading to truncation of 103 amino acids at the N-terminal of the SMARCAL1 protein. The truncated 103 amino acids contain the putative nuclear location signal (NLS; Figure 3A and C) [13].

The maternal mutation, c.1682G>A (p.Arg561His), occurred in the functionally important region of motif IIa in the SNF2-related domain (Figure 3B, C) [14] involved in ATP hydrolyzation and DNA binding. As this mutation replaces basic arginine (isoelectric point 10.76) with histidine (isoelectric point 7.59), it is predicted to disrupt the local spatial structure of the wild-type protein, although topological simulation did not indicate any gross change. Hence both mutations are pathological and predicted to result in serious loss of normal function of the wild-type protein.

Discussion

SIOD is an autosomal recessive pleiotropic disorder caused by mutations in the SMARCALI gene. This gene encodes HHARP which is a member of the SNF2 family of ATP-driven molecular motor proteins, the DNA-dependent ATPases [15]. This protein is known as an ATP-dependent annealing helicase, which rewinds single-stranded DNA bubbles that are stably bound by replication protein A [6]. It is therefore involved in a wide range of biological functions. The severity and diversity of the phenotypes caused by its defects are therefore unsurprising. The phenotype of the boy reported in this paper was severe and involved several systems typical of SIOD. Interestingly, our 8-year-old patient also exhibited a grossly retarded carpal bone age of only 4 years. This degree of bone age retardation has not previously been reported in the literature. Such gross retardation cannot be explained by growth hormone deficiency, as the stimulating test result was normal. It is unclear whether it is caused by some other cause such as malnutrition, or is specifically related to the novel mutations identified.

To date more than 44 mutations have been identified in the SMARCALI gene, with 20 missense mutations discovered in analysed patients [16]. The significance of truncating mutations losing a functionally important domain is obvious, but the clinical implications and significance of missense mutations are less clear. In 2002 Boerkkoel et al. suggested that missense mutations cause milder SIOD by allowing retention of partial HHARP function [5]. Expression of SMARCALI missense mutants in Drosophila showed that disease severity is inversely proportionate to overall SMARCAL1 activity [13]. However, in 2007, Clewling et al. pointed out that severe SIOD could be caused by biallelic missense mutations in the SMARCAL1 gene [7], suggesting that genotype–phenotype correlations remain largely unpredictable.

The paternal mutation, c.3G>A (p.Met1?), was predicted to introduce an alternative ORF with an initiation codon (ATG) present 310 bp downstream. Similar mutations in the initial codon have been reported to be associated with several genetic diseases [17], especially those that influence the ORF [18]. Moreover, the alternative

Table 1. Disease severity score of the patient according to Clewling et al. [7]

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear growth failure before 10 years of age</td>
<td>1</td>
</tr>
<tr>
<td>Renal failure</td>
<td>1</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>1</td>
</tr>
<tr>
<td>Recurrent infections</td>
<td>1</td>
</tr>
<tr>
<td>Cerebral ischaemia</td>
<td>0</td>
</tr>
<tr>
<td>Death before 10 years of age</td>
<td>2</td>
</tr>
<tr>
<td>Death after 10 years of age</td>
<td>0</td>
</tr>
<tr>
<td>Total score</td>
<td>6</td>
</tr>
</tbody>
</table>

Fig. 2. Genetic analysis of the family. (A) Pedigree, haplotype and mutation analysis of the family. Coloured boxes indicate the mutation-containing haplotypes. The father carries the c.3G>A mutation and the mother the c.1682G>A mutation. The child inherited mutant haplotypes from both parents. (B and C). Partial nucleotide sequences of exon 3 of the SMARCALI gene in the patient and in the father. The forward (B) and reverse (C) sequences show the change at position c.3G>A. Arrows indicate the position of the mutation, and the underline denotes the codon containing the mutation. (D and E). Partial nucleotide sequences of exon 10 of the SMARCALI gene in the patient and in the mother. The forward (D) and reverse (E) sequences show the change at position c.1682G>A. Arrows indicate the position of the mutation, and the underline denotes the codon containing the mutation.
ORF was predicted to truncate 103 amino acids which include a putative NLS at the N-terminal of the SMARCAL1 protein. Therefore, the mutant protein would have inappropriate subcellular localizations [19].

In the compound heterozygote situation described here, the missense maternal mutation, c.1682G>A (p.Arg561His), is located in the SNF2-related domain of the protein, which has altogether seven motifs homologous with motifs found in other helicases [20]. The SNF2-related domain contains a nucleotide-binding site, a phosphate-binding loop (also called Walker A and B boxes), a DEAD box and DNA- and ATP-binding motifs [21]. The arginine at the 561st amino acid position is highly conserved in all the species examined. The p.Arg561His mutation therefore affects a remarkably conserved motif, one flanking the Walker B box [14] involved in ATP hydrolyzation and DNA binding. Furthermore, homology of a different substitution at the same site, p.Arg561Cys, has been reported to be associated with mild SIOD [22]. These data together suggest a critical role for this residue in normal SMARCAL1 function. The change in isoelectric point of the 561st amino acid from 10.76 (arginine) to 7.59 (histidine) is predicted to alter the local spatial structure of the protein.

In summary, we report the first severe Chinese SIOD patient with distinct phenotype (a markedly retarded bone age) and two novel mutations in the SMARCAL1 gene.

Acknowledgements. The authors thank the parents of the patient for giving their consent for publication of Figure 1 and all the volunteers who donated blood samples for this study. We thank Prof. Du Minglian for clinical consultation. We would also like to thank Dr Dorian Pritchard for his help in manuscript preparation and Drs Q. Pan-Hammarström and L. Hammarström for helpful comments on the manuscript. This project was supported by the Natural Science Foundation of Guangdong Province (Grant No. 06300772), the Medical Science and Technology Foundation of Guangdong Province (Grant No. B2006034) and the 985 Project of China, Phase II.

Conflict of interest statement. None declared.

References