The single nucleotide polymorphisms in Smad-interacting protein 1 gene contribute to its ectopic expression and susceptibility in Hirschsprung’s disease☆

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Abstract

Article history:
Received 30 January 2014
Available online 24 February 2014

Keywords:
Hirschsprung’s disease
Smad-interacting protein 1
Single nucleotide polymorphisms
Susceptibility

Hirschsprung’s disease (HSCR) is the third most common congenital disorder of the gastrointestinal tract. It is an anomalous enteric nervous system (ENS) characterized by the absence of ganglion cells in the myenteric and submucosal plexuses. It has been reported that the Smad-interacting protein 1 (SIP1) is critical in embryonic development of ENS for its regulation on neural crest cells. In the present study, we analyzed 3 polymorphisms of the SIP1 gene rs41292293 (exon5), rs34961586 (exon6) and rs13017697 (exon8) to determine their potential contributions to the susceptibility of HSCR. Allele frequencies and genotype distributions were analyzed by sequence analysis in 107 HSCR patients and 107 normal controls. The SIP1 expression was carried out by using real-time PCR, western blot and immunohistochemistry. Polymorphic analysis indicated that the genotype distributions and allele frequencies in SIP1 gene rs41292293, rs34961586 and rs13017697 were statistically different between HSCR and normal controls. The expression analysis revealed that SIP1 was ectopically expressed in the aganglionic segments; neither the mRNA nor the protein levels demonstrated that the difference compared with those in the normal segments. In conclusion, the single nucleotide polymorphisms in SIP1 gene rs41292293, rs34961586 and rs13017697 are associated with the ectopic expression of this gene in human HSCR and contribute to the susceptibility of this disease in population.

Introduction

Hirschsprung’s disease (HSCR) is one of the most common congenital malformations of enteric nervous system (ENS) in children. It is the third most common congenital disorder of the gastrointestinal tract worldwide, and it occurs in 1/5000 live births (Barlow et al., 2012; Burzynski et al., 2009; Cacheux et al., 2001). HSCR is characterized by the absence of ganglion cells in the myenteric and submucosal plexuses of the gastrointestinal tract, resulting in intestinal obstruction and constipation in neonates and children (Burzynski et al., 2009). It is caused by an anomalous enteric nervous system and is therefore considered to be a neurocristopathy. It is well established that HSCR is a set of complex diseases with extensive molecular genetics bases. At least 10 different genes and 5 chromosomal loci have been proven to contribute to its pathogenesis. The most commonly candidate genes involved in HSCR include RET, GDNF, Sox10, GFRα1, and EDNRB (Carlson et al., 2003; Cerruti Mainardi et al., 2004). Among these well established genes, the SIPI gene has turned to be critical because of its roles in migration and survival of neural crest cells (NCCs). SIPI (Smad-interacting protein 1) is a Smad-interactive multiple zinc finger structured protein and it possesses a specific DNA binding domain. It is encoded by the ZFHX1B gene that consists of 10 exons and is located in 2q22 (Chang-qing, 2004; Garavelli et al., 2003; Gibbs and Singleton, 2006). In HSCR patients, the ZFHX1B gene has been found to have a mutation in the exon 8 which causes a premature stop codon that truncated the SIPI protein. Mutation of the ZFHX1B gene usually occurs at exon 3, exon 4, exon 5, exon 6 and exon 8 (Gregory-Evans et al., 2004). However, currently there was no report of SIPI gene polymorphism. To determine whether single nucleotide polymorphisms (SNPs) in the SIPI gene are associated with HSCR, we compared the SNPs in the rs41292293 (exon 5), rs34961586 (exon 6) and rs13017697 (exon 8) sequence of the SIPI gene between HSCR patients and healthy persons in China.

☆ All the work was performed in Shengjing Hospital of the China Medical University; the study was supported by the National Natural Science Foundation of China (No. 30700917) and the Science Research Project of Liaoning Key Laboratory of Congenital Malformations (No. L2013290); the manuscript has not been presented at any scientific meeting.

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http://dx.doi.org/10.1016/j.yexmp.2014.02.004
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Materials and methods

Patients

Blood samples of 107 HSCR patients from a regional hospital (Shengjing Hospital of China Medical University) in Shenyang city, China were used in this study. The definition of the HSCR cases was patients who had a history of delayed meconium evacuation after birth and recurrences at later of birth, a clinical manifestation of stenosis of the colon in barium enema, and lack of ganglion cells in the transitional, expansion, rectal mucosa, anorectal manometry and postoperative pathologic section by acetylcholinesterase histochemistry. Patients with familial obstipation and other congenital GI tract malformation histories were excluded from the study. The patients ranged from 6 months to 13 years old including 79 male and 28 female. Blood of 107 healthy children that matched with the HSCR group in ages and genders was used as controls.

Reagents and instruments

Taq DNA polymerase, MarkerDL, 2000, dX174-Hinc II were purchased from TaKaRa Biotechnology (Dalian, Liaonin Province, China). Primers were synthesized by Invitrogen (Shanghai, China). Restriction endonucleases were products of Fermentas (Latvia). A Biometra thermal cycler was purchased from Biometra Biomedizinische Analytik GmbH (Goettingen, Germany).

Genomic DNA extraction

200 μl peripheral blood from each patient or healthy person was collected in a Vacutainer EDTA anticoagulation tube. Blood DNA was extracted using the QIAamp DNA Blood Mini Kit. DNA concentrations were determined with UV spectrophotometer, and then DNA integrity and purity were assayed in 1.5% agarose gel electrophoresis. Blood DNA was kept at −70 °C until use.

PCR amplification and restriction enzyme digestion of PCR products

Primers specific to SIP1 gene segments rs41292293, rs34961586 and rs13017697 were designed using the DNASTAR program and synthesized by Invitrogen (Shanghai) (Table 1). The primers have no homology with other genes as determined by BLAST analysis on homology. PCR amplification conditions were shown in Table 1. rs34961586, rs41292293 and rs13017697 were C/G and A/G-rich templates and contained restriction sites for BstN I (CG↓ GG), Dsa I (C↓ CGTC) and Ddel (C↓ TCAG), respectively. The PCR products were digested with restriction enzymes as DsaI for rs41292293, BstN I for rs34961586, and Dde I for rs13017697. DNA was electrophoresed on 2–2.5% agarose gel, stained with ethidium bromide (EB), and visualized with an automatic gel documentation system (TaKaRa Biotechnology Co., Ltd.).

Sequencing of PCR product

Purified PCR products were sequenced with an automatic DNA sequencer by Invitrogen (Shanghai, China). Sequences of rs41292293, rs34961586 and rs13017697 obtained from the patients were compared to the sequences of patients and healthy persons in the NCBI database by BLAST, to identify the mutation points. Genetic sequences with existing mutated points/loci were reverse-sequenced for confirmation.

Immunohistochemistry

The frozen tissue was incubated in PBS for 10 min and subsequently transferred to a solution of 4% paraformaldehyde in PBS for 2 h at room temperature, followed by tissue embedding and sectioning. After washing, the slides were incubated in 1% Triton X-100 overnight at 4 °C. The slides were subsequently incubated in 0.5% Triton X-100 + 2% normal serum (NS, Zymed 50-197, Beijing, China) + 1% BSA in PBS overnight at 4 °C. The primary antibody was used at a dilution of 1:200 in 0.2% Triton + 1% BSA + 1% NS in PBS. After incubation for 24 h at 4 °C, the samples were washed with PBS for 3 × 5 min at room temperature and incubated with the appropriate secondary antibody at a dilution of 1:50 in 1% NS for 2 h at room temperature, followed by washing in PBS for 3 × 5 min. Finally, the slides were mounted with SlowFade antifading mounting media, and the preparations were visualized with a microscope.

Western blot analysis

Protein extract samples 100 μg in size were separated by performing 12.5% SDS-PAGE and were subsequently transferred in Tris–HCl methanol (20 mM Tris, 150 mM glycine, 20% methanol) onto polyvinylidene difluoride membranes (Millipore, USA) using a trans-blot electrophoresis transfer cell (Bio-Rad). The blot was probed with antibodies against SIP1 or actin (Santa Cruz Biotechnology). Antigen–antibody complexes were visualized using enhanced chemiluminescence reagents (GE healthcare). Detected bands were quantified using the Gel-pro 4.0 software (Media Cybernetics, LP).

Real-time quantitative PCR

Total RNA was extracted from the frozen tissues using the Trizol reagent (Invitrogen) according to the manufacturer's protocol. cDNA synthesis was performed using 3 μg of RNA with the TaKaRa RNA PCR kit (Takara). Real-time PCR amplifications were performed in triplicate on a Light Cycler with the primer 5′-AGGCATATGGTGACCAAA-3′ and 5′-CTTGAATCCGGTTACCTGC-3′. The housekeeping gene ß-actin (Takara DR3783) served as an endogenous control. The relative mRNA levels for each sample were calculated via the 2−ΔΔCt method.

Statistical analysis

The χ2 test was performed to determine whether each polymorphism was in the Hardy–Weinberg equilibrium within the control and patient groups. The relative density of the bands was expressed as the 2−ΔΔCt value of each sample as parametric data for quantitative real-time PCR, western blot analysis and immunohistochemistry. Statistical significance was determined using the Student's t-test; a P-value <0.05 was considered to indicate a statistically significant difference.

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Table 1

<table>
<thead>
<tr>
<th>SNP locus</th>
<th>Primer sequence (5′-3′)</th>
<th>Length</th>
<th>Location</th>
<th>Endonuclease</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs41292293</td>
<td>F: AGG ATC AAG GCA CGG GAG GAC R: GGG CAT TAA AGG GAG GTG ACA GG</td>
<td>365 bp</td>
<td>A/G, exon5</td>
<td>Dsa I</td>
</tr>
<tr>
<td>rs34961586</td>
<td>F: CTC GCC TAC AGA CCT GCC ACC T R: TGG CCA ATC AAA GCA ATA TCG TTT C</td>
<td>298 bp</td>
<td>C/G, exon6</td>
<td>BstN1</td>
</tr>
<tr>
<td>rs13017697</td>
<td>F: AGA CCG ACA GGC GGA ATC ATA R: AAG TGT AGG GAT GGA AGC</td>
<td>222 bp</td>
<td>A/G, exon8</td>
<td>Dde I</td>
</tr>
</tbody>
</table>
Results

PCR amplification of the SIP1 gene

PCR amplification was successfully performed. The amplified segments of the SIP1 gene were 222 bp, 265 bp and 292 bp which were in accordance with theoretical lengths. The amount of amplified products was large and no non-specific bands appeared (Fig. 1).

Distribution of SIP1 allele and genotype frequencies in patients with HSCR and controls

Genotype distributions in the 3 SNPs were in accordance with the Hardy-Weinberg equilibrium (Figs. 2, 3). As illustrated in Table 2 that GA and GG genotype frequencies in rs41292293 were significantly different between the HSCR patients and healthy persons ($P < 0.05$). The frequencies of A and G alleles were also significantly different between the HSCR patients and healthy persons ($P < 0.05$) (Fig. 2A). G is more common in the HSCR patient, suggesting a significant association of allele G with HSCR. In rs34961586, the frequencies of GC and GG genotypes were significantly different between the HSCR group and the control group ($P < 0.05$). The GC genotype was more abundant in HSCR patients (Table 2). The frequencies of C and G alleles were also significantly different ($P < 0.05$) between the HSCR and the healthy group (Fig. 2B), suggesting a significant association of allele C with HSCR.

In rs13017697, the frequencies of AG and GG genotypes and A and G alleles were significantly different ($P < 0.05$) between the HSCR group and healthy group. GG genotype was more abundant than AA genotype in the HSCR patients (Table 2). The frequencies of C and G alleles were also significantly different ($P < 0.05$) between the HSCR patients and healthy persons ($P < 0.05$) (Fig. 2A). G is more common in the HSCR patient, suggesting a significant association of allele G with HSCR. In rs34961586, the frequencies of GC and GG genotypes and A and G alleles were significantly different ($P < 0.05$) between the HSCR group and healthy group. GG genotype was more abundant than AA genotype in the HSCR patients (Table 2, Fig. 2C), suggesting a significant association of allele G with HSCR. In rs13017697, the frequencies of AG and GG genotypes and A and G alleles were significantly different ($P < 0.05$) between the HSCR group and healthy group. GG genotype was more abundant than AA genotype in the HSCR patients (Table 2). The frequencies of C and G alleles were also significantly different ($P < 0.05$) between the HSCR patients and healthy persons ($P < 0.05$) (Fig. 2A). G is more common in the HSCR patient, suggesting a significant association of allele G with HSCR. In rs34961586, the frequencies of GC and GG genotypes and A and G alleles were significantly different ($P < 0.05$) between the HSCR group and healthy group. GG genotype was more abundant than AA genotype in the HSCR patients (Table 2). The frequencies of C and G alleles were also significantly different ($P < 0.05$) between the HSCR patients and healthy persons ($P < 0.05$) (Fig. 2A). G is more common in the HSCR patient, suggesting a significant association of allele G with HSCR. In rs13017697, the frequencies of AG and GG genotypes and A and G alleles were significantly different ($P < 0.05$) between the HSCR group and healthy group. GG genotype was more abundant than AA genotype in the HSCR patients (Table 2). The frequencies of C and G alleles were also significantly different ($P < 0.05$) between the HSCR patients and healthy persons ($P < 0.05$) (Fig. 2A). G is more common in the HSCR patient, suggesting a significant association of allele G with HSCR. In rs34961586, the frequencies of GC and GG genotypes and A and G alleles were significantly different ($P < 0.05$) between the HSCR group and healthy group. GG genotype was more abundant than AA genotype in the HSCR patients (Table 2). The frequencies of C and G alleles were also significantly different ($P < 0.05$) between the HSCR patients and healthy persons ($P < 0.05$) (Fig. 2A). G is more common in the HSCR patient, suggesting a significant association of allele G with HSCR. In rs13017697, the frequencies of AG and GG genotypes and A and G alleles were significantly different ($P < 0.05$) between the HSCR group and healthy group. GG genotype was more abundant than AA genotype in the HSCR patients (Table 2). The frequencies of C and G alleles were also significantly different ($P < 0.05$) between the HSCR patients and healthy persons ($P < 0.05$) (Fig. 2A). G is more common in the HSCR patient, suggesting a significant association of allele G with HSCR. In rs34961586, the frequencies of GC and GG genotypes and A and G alleles were significantly different ($P < 0.05$) between the HSCR group and healthy group. GG genotype was more abundant than AA genotype in the HSCR patients (Table 2). The frequencies of C and G alleles were also significantly different ($P < 0.05$) between the HSCR patients and healthy persons ($P < 0.05$) (Fig. 2A). G is more common in the HSCR patient, suggesting a significant association of allele G with HSCR. In rs13017697, the frequencies of AG and GG genotypes and A and G alleles were significantly different ($P < 0.05$) between the HSCR group and healthy group. GG genotype was more abundant than AA genotype in the HSCR patients (Table 2). The frequencies of C and G alleles were also significantly different ($P < 0.05$) between the HSCR patients and healthy persons ($P < 0.05$) (Fig. 2A). G is more common in the HSCR patient, suggesting a significant association of allele G with HSCR.

Expression level of SIP1 in the aganglionic segment in HSCR

The mRNA and protein levels of SIP1 in the aganglionic and normal segment were quantitatively determined by real-time quantitative PCR and western blot. The results revealed that both the mRNA and protein levels were slightly higher in the aganglionic segment than in the control group, but the difference was not statistically significant.

Discussion

SIP1, also known as ZEB2, for zinc finger E-box-binding protein 2 and ZFHX1B, belongs to the EF-1 or ZEB protein family (Chang-qing, 2004; Garavelli et al., 2003; Gibbs and Singleton, 2006; Gregory-Evans et al., 2004). These proteins are characterized by a homeodomain flanked by two separated, highly conserved zinc finger clusters: an N-terminal and a C-terminal one, which contain four and three zinc fingers, respectively. Each zinc finger cluster can bind independently to CACCT(G) sequences present in the promoter regions of genes involved in differentiation and development, such as the Xenopus Xbra2 promoter, the human 4-integrin promoter and the E-cadherin promoter (Chang-qing, 2004). The integrity of the two zinc finger clusters of SIP1 is necessary for its binding as a monomer to the target promoter sequences. SIP1 acts as a transcriptional repressor and contains consensus binding sites for the co-repressor CtBP. Gene repression by SIP1 has been reported to occur both dependent on and independent of a CtBP corepressor complex (Chang-qing, 2004; Garavelli et al., 2003; Gibbs and Singleton, 2006; Gregory-Evans et al., 2004). Recently it was reported that the SIP1 protein is associated with epithelial mesenchymal transitions (EMT) during development and neural crest cell formation. Loss of SIP1 expression was correlated with loss of the migratory capacities of neural crest cells and resulted in a deficiency of ENS formation (Chang-qing, 2004; Gibbs and Singleton, 2006; Hegarty et al., 2013; Kenny et al., 2010). Therefore, mutations in the SIP1 gene are the most relevant and most important etiology of HSCR. Among the 10 exons, genetic mutations of exon 3 and exon 8 were associated with HSCR.
combined with intelligence development retardation, microcephaly and facial deformity (Hegarty et al., 2013; Kenny et al., 2010; Miquelajauregui et al., 2007; Mutsuddi et al., 2006; Obermayr et al., 2012). However, there were no similar reports regarding these mutations in the other exons. Thus, we selected three SIP1 loci to study the correlation between the mutation of the gene and HSCR.

At present, the mutation of SIP1 in HSCR subjects is decentralized and lacks hotspot. Minority of patients miss a large fragment of the

**Fig. 3.** (A) Sequencing of rs41292293. a: GA genotype, C → resulting loss of restriction site; b: GC genotype, C → resulting loss of restriction site. Arrows, nucleotide variations; underlined sequences, mutated Dsa I recognition sites (C↓GTC). (B) Sequencing of rs34961586. a: GC genotype, C → resulting loss of restriction site. b: GC genotype, G → resulting loss of restriction site. c: GC genotype, C → resulting loss of restriction site. Arrows, nucleotide variation; underlined sequences, mutated Dsa I recognition sites (CC↓AGG). (C) Sequencing of rs13017697. a: AG genotype, T → resulting loss of restriction site. b: GG genotype, G → resulting loss of restriction site. Arrows, nucleotide variations; underlined sequences, mutated Dsa I restriction sites (C↓CAG).

**Table 2**

Hardy–Weinberg equilibrium analysis and the relative risks of rs41292293, rs34961586 and rs13017697 alleles and genotypes.

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Frequency of Type</th>
<th>Type</th>
<th>HSCR</th>
<th>Control</th>
<th>$X^2$</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs41292293</td>
<td>Genotype (%)</td>
<td>GA</td>
<td>46 (42.99)</td>
<td>45 (42.05)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>32 (29.91)</td>
<td>56 (52.34)</td>
<td>3.662</td>
<td>0.056</td>
<td>0.559 (0.307–1.016)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>29 (27.1)</td>
<td>6 (5.61)</td>
<td>10.951</td>
<td>0.001</td>
<td>4.728 (1.792–12.48)</td>
</tr>
<tr>
<td></td>
<td>Allele (%)</td>
<td>G</td>
<td>104 (48.6)</td>
<td>57 (26.64)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>110 (51.4)</td>
<td>157 (73.4)</td>
<td>21.994</td>
<td>0.000</td>
<td>2.604 (1.738–3.903)</td>
</tr>
<tr>
<td>rs34961586</td>
<td>Genotype (%)</td>
<td>GC</td>
<td>48 (44.86)</td>
<td>39 (36.45)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>37 (34.58)</td>
<td>60 (56.07)</td>
<td>5.350</td>
<td>0.021</td>
<td>0.501 (0.278–0.903)</td>
</tr>
<tr>
<td></td>
<td>Allele (%)</td>
<td>G</td>
<td>124 (57.0)</td>
<td>159 (74.3)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>98 (42.99)</td>
<td>55 (25.7)</td>
<td>14.185</td>
<td>0.000</td>
<td>2.180 (1.448–3.281)</td>
</tr>
<tr>
<td>rs13017697</td>
<td>Genotype (%)</td>
<td>AG</td>
<td>42 (29.25)</td>
<td>56 (52.34)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>63 (58.88)</td>
<td>43 (40.18)</td>
<td>5.602</td>
<td>0.018</td>
<td>0.512 (0.293–0.894)</td>
</tr>
<tr>
<td></td>
<td>Allele (%)</td>
<td>G</td>
<td>166 (78.5)</td>
<td>72 (33.04)</td>
<td>87.421</td>
<td>0.000</td>
<td>0.139 (0.080–0.214)</td>
</tr>
</tbody>
</table>

All 3 loci were consistent to Hardy–Weinberg equilibrium law analysis. The genotype frequency of HSCR vs controls, A: $X^2 = 21.671, P = 0.000$. B: $X^2 = 12.918, P = 0.002$. C: $X^2 = 9.374, P = 0.009$. 

gene, whereas majority of patients were point mutations, missense, nonsense, and frame shift mutations (Miquelajauregui et al., 2007; Mutsuddi et al., 2006; Obermayr et al., 2012). In recent years, large-scale studies on SNPs revealed that single nucleotide variation may provide new methods in studying complex genetic diseases, individual prevalence risks and drug reaction differences. In the entire human population, there are about 15 millions of SNP loci (rare SNP loci frequency consisted of at least 1%). About every 300–600 bp at average, there is one base mutation (Rogers et al., 2013). SNPs are the genetic markers of disease susceptibility in human genomes, or may even be the susceptible gene loci that directly lead to common diseases. With the completion of the Human Genome Project, how to utilize human genome SNP information in exploring hereditary characteristics, especially for the genetic mechanisms of complex diseases and drug actions, has become a research hotspot in recent years. The most common DNA variation is a single base difference, which can be transition or transversion, single nucleotide insertion or deletion. Genomes which have a variation occurrence rate of higher than 1% are known as SNPs. For example: some humans’ chromosome has an A base on a specific locus, while on other humans’ chromosome, a G base is present on the same locus. Each base type on the same location is called an allele. Each human’s chromosomes have two sets of alleles, except for sex chromosomes. A pair of allele types in a human chromosome is known as genotype. The term ‘genotype’ can be referred to a certain SNP allele, and may also be referred to multiple SNP alleles in a genome (Sasongko et al., 2007; Van de Putte et al., 2003). Each person’s genotype may have 3 possible pairs, which are AA, AG or GG. Verification of an individual genotype is known as genotyping. Currently, many high-throughput SNP analysis techniques were used in SNP genotyping, which have greatly accelerated the growth of SNP data. Any observed physical or physiological quality of an organism (human) generated by the interaction of genotype and environmental differences is called phenotype. The search of correlation between genotype and phenotype has been the basic goal of all genetic studies.

In the present study, we assayed the polymorphism of the gene loci using whole blood specimens from HSCR patients and 107 healthy children without any blood relationship from the north of China. Through studying HSCR self-SIP1 gene polymorphism, we proved that SIP1 SNPs are varied in HSCR and led to an ectopic expression of this gene, indicating that the genetic background of HSCR itself was very essential for the development of SIP1 exon 5, exon 6, and exon 8. The results were consistent with those of previous studies that SIP1 was mutated in HSCR (Hegarty et al., 2013; Kenny et al., 2010; Miquelajauregui et al., 2007; Mutsuddi et al., 2006; Obermayr et al., 2012). This is first genetic distribution data regarding SIP1 rs34961586, rs41292293 and rs13017697 gene polymorphisms conducted on children from the population of Northern China, and this may provide references for future study researches on HSCR. However, this method also had some limitations because the function of a single SNPs itself was limited, and the range of other SNPs to this locus linkage imbalance was also limited. Therefore a single SNP cannot represent other candidate genes in the development of a disease. In addition, majority of SNPs have only two alleles, forming 3 genotypes, therefore a single SNP can only provide minimal amount of information. By selecting several SNPs from a candidate gene for an associated study, may even better support the determination of a candidate gene or its adjacent chromosomal region involved in a disease pathogenesis, or by making a haplotype as a disease-associated genetic marker (Wakamatsu et al., 2001; Wu et al., 2005). Therefore, studying the combination differences of genotypes may help us to understand the pathogenesis of HSCR. We have focused on...
the distribution of a partial SIP1 gene polymorphism of children in Northern China and normal healthy subjects and its association with HSCR. In order to make a clear understanding of SIP1 gene polymorphism and HSCR relevance in Chinese population, it is necessary to further conduct analysis on the rest of SIP1 gene loci and on a large scale of population. Using case–control analysis of regional SNPs, it may clarify the relationship between SIP1 gene SNPs and abnormal phenotypes (Yamada et al., 2001). With the progress of the Human Genome Project, we believe that such polymorphism in genomes may help to explain individual phenotype variation, different population and individual susceptibility towards HSCR.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

References
