Research paper

Novel mutations in SKIV2L and TTC37 genes in Malaysian children with trichohepatoenteric syndrome

Way Seah Lee, Kai Ming Teo, Ruey Terng Ng, Sze Yee Chong, Boon Pin Kee, Kek Heng Chua

Abstract

Trichohepatoenteric syndrome (THES) is a rare autosomal recessive disorder that is classically associated with intractable diarrhea with an onset within the first few months of life. Herein, we investigated and reported novel mutations in two causal genes in 3 Malaysian cases. Genomic DNA was extracted from peripheral blood obtained from patients in two Malaysian Chinese families. The exons of SKIV2L and TTC37 genes were amplified and sequenced by bi-directional sequencing to identify the point mutations within the coding sequence. Three Chinese boys from two families with characteristic features and clinical course were diagnosed with THES. In family-1, two point mutations were identified in the SKIV2L gene (c.1891G>T and c.3187C>T). In family-2, a single-nucleotide duplication (c.3426dupA) was found in the TTC37 gene. These mutations cause the production of abnormal non-functional gene product leading to the clinical manifestations in the patients. We reported three point mutations, which have not been previously described in other patients with THES in SKIV2L and TTC37 genes, including one nonsense, one frameshift, and one missense mutations.

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1. Introduction

Most of the diarrheal diseases occurring in children are infectious in origin and are self-limiting. However, a small minority of young infants may suffer from rare, inheritable causes of persistent diarrhea, which include microvillus inclusion disease, tufting enteropathy, congenital chloridorrhea, congenital sodium diarrhea and intractable diarrhea with phenotypic abnormalities (Lee and Boey, 1999; Lee et al., 2008; Pezzella et al., 2013).

First described by Stankler et al. in 1982, intractable diarrhea with phenotypic abnormalities is also known as phenotypic diarrhea of infancy and trichohepatoenteric syndrome (THES), as coined by Verloes and co-workers (Stankler et al., 1982; Girault et al., 1994; Verloes et al., 1997). The main clinical features of THES include facial dysmorphism (prominent forehead and cheeks, broad nasal bridge and hypertelorism), trichorrhexis nodosa, severe and persistent diarrhea leading to severe malabsorption and failure to thrive (FTT) (Goulet et al., 2008). There may be fibrosis or cirrhosis of the liver, functional T-cell immune deficiency, and defective antibody production (Goulet et al., 2008).

THES is an autosomal recessive inherited disorder and is often associated with mutations in the genes superkiller viralicidic activity 2 (SKIV2L) and tetratricopeptide repeat domain-containing protein 37 (TTC37) (Hartley et al., 2010; Fabre et al., 2011; Fabre et al., 2012). The SKIV2L gene consists of 28 exons and is approximately 11 kb in size. It has been mapped to chromosome 6p21.33 (Lee et al., 1995; Yang et al., 1998). The gene transcription yields a 137 kDa RNA helicase, containing 1246 amino acid (Dangel et al., 1995). TTC37 is located on chromosome 5q15 and contains 43 exons and spans 91,113 bases and encodes a putative protein known as thespin (Blatch and Lassle, 1999; Fabre et al., 2011). The spin is formed by 1564 amino acid residues with 20 TRP domains, which are incorporated into binding grooves which may serve the purpose for protein–protein interaction (Blatch and Lassle, 1999). The function of these gene products in human is not fully determined. Proteins of SKIV2L and TTC37 genes are ortholog respectively to the Ski2p and Ski3p of the yeast system (Brown et al., 2000). Together with two copies of Ski8p, these proteins form the superkiller (Ski) complex that functions as the cofactor of cytosolic exosome is responsible for degradation of aberrant mRNAs (Synowsky and Heck, 2008). Previous reports have demonstrated that exosome complex could be a target of the host’s autoimmunity (Staals and Pruijn, 2010). Therefore, it is plausible to assume a direct pathogenic effect from the malfunctioned/malformed exosome-cofactor caused by the nucleotide sequence changes could lead to various clinical manifestations in patients with THES.
Most of the earlier reports on the clinical manifestations and muta-
tional analysis of THES were from patients originated from the Middle
East, Indian Subcontinent, or Western Europe (Stankler et al., 1982;
Hartley et al., 2010; Fabre et al., 2011; Fabre et al., 2012; Kotecha et al.,
2012; Fabre et al., 2013; Fabre et al., 2014). Recently, a group of re-
searchers from Singapore and Hong Kong had also reported two cases
of THES in two Chinese children (Chong et al., 2015). In the present
study, we reported three young Chinese children with classical features
and clinical course of THES, harboring novel mutations in two known
disease genes for THES.

2. Materials and methods

2.1. Patient recruitment

Patients who were diagnosed with THES were retrieved from the da-
tabase of the Department of Paediatrics, University Malaya Medical Cen-
ter (UMMC), Kuala Lumpur, Malaysia. The present study was approved
by the UMMC ethics committee. Parental consent was obtained prior to
blood sample collection for mutational analysis. The clinical course of
the disease, including clinical presentation and treatment outcome,
were noted.

2.2. Molecular genetic studies

Genomic DNA was extracted from the blood samples according to
the previous published approach (Puah et al., 2007; Tan et al., 2010;
Chua et al., 2011). The quality and quantity of the DNA samples were
evaluated with a Nanophotometer (Implen GmbH, Germany). Mutation
screening was conducted on genomic samples by bi-directional DNA re-
sequencing of the SKIV2L and TTC37 genes. Primer sets were designed to
span all exons (except the non-coding exon-1, exon-2, and exon-3 of
TTC37 gene), including the intron–exon gap, with amplicon length of
not more than 1000 bp. The resulted sequences were aligned and com-
pared with the human genome reference (hg19) and variations from
the reference were recorded via BioEdit and Sequencher software. The
nomenclature of point mutations was carried out in accordance to the
regulations by Human Genome Variation Society. All point mutations
were further validated by repeating the examination. The impact of
point mutations on the proteins of SKIV2L and TTC37 genes was predict-
ed using computational approach via Polyphen-2 and SIFT (Kumar et al.,
2009; Adzhubei et al., 2010). To determine the frequency of the point
mutations in healthy local population, a screening on 200 ethnic-
matched (Malaysian Chinese) individuals was conducted via High Res-
olution Melting (HRM) analysis (for SKIV2L c.1891G>A) and PCR-
resequencing approach (for SKIV2L c.3187C>T and TTC37 c.3426dupA).

Fig. 1. Pedigrees of the two subject families investigated in the present study. (a) Family-1 with two affected brothers. (b) Family-2 with only one child who is affected by THES.
3. Results

3.1. Clinical studies

Family-1: LF, an 8-year-old Chinese boy, was delivered to healthy, non-consanguineous parents. He has an older sister who is currently well, and a younger brother (LL) who was also affected (Fig. 1). LF had persistent diarrhea, marked FTT at the first month of age and was initially diagnosed as having multiple protein allergy (Table 1). He was extensively investigated, which included a jejunal biopsy showing severe villous atrophy. The stomach and colon were normal macroscopically and histologically. Other investigations included a negative sweat test (chloride content: 35 mmol/L) and a negative serology for celiac disease. He developed diarrhea with cow milk and soy formula. The diarrhea stopped when amino acid-based formula was started. Despite this, there was persistent severe FTT.

Clinically he had severe FTT (both weight-for-age and height-for-age were less than 3 percentile). He had dysmorphic features with a prominent forehead and depressed nasal bridge (Fig. 2). The hair was sparse, depigmented, easily removable and unmanageable. However microscopically, there was no evidence of trichorrhexis nodosa. There was no palpable liver and the liver enzymes were within normal range. The immunoglobulin IgG was within normal limit, but he has raised IgA and IgM levels (Table 1). However, clinically there was no frequent infection. Echocardiographic examination showed atrial septal defect and bicuspid aortic valve but clinically he was not in cardiac failure.

He had several episodes of pneumonia and acute gastroenteritis with dehydration up to the age of four years old. Subsequently he had been well. Currently he is attending mainstream school but is slow in his school work. He never requires parenteral nutrition and is currently on a normal diet. He has no diarrhea and is growing below third centile for both weight and height-for-age.

LL is the younger brother of LF. He developed severe diarrhea and dehydration at day 10 after birth. He needed a period of parenteral nutrition but was subsequently changed to amino acid-based formula. He also had several episodes of infections (acute gastroenteritis, pneumonia) in the first twelve months of age. His facial appearance resembles his elder brother with prominent forehead, and depressed nasal bridge (Fig. 2). Macroscopically the hair looked normal. He is on normal diet with caloric supplement. His latest growth parameters (both weight and height-for-age) were markedly below third percentile (Supplementary data). The antibody level for this patient was not determined.

Family-2: CHT was delivered at 31 weeks of gestation to elderly parents, with a birth weight of 1.1 kg. Family pedigree is shown in Fig. 1. He developed persistent diarrhea at the age of two months (Table 1). The diarrhea persisted despite frequent change of formula. He was referred to UMMC for management at the age of 13 months. Clinically his facial appearance was characteristics of those children with THES: facial dysmorphism, prominent forehead and depressed nasal bridge. The hair was also characteristic of those seen in THES.

Assessment of the cardiovascular system included an echocardiography which showed a normal cardiac anatomy. The complement C3 and

### Table 1

Summary of clinical signs of three Chinese children with trichohepatoenteric syndrome.

<table>
<thead>
<tr>
<th>Patient identification</th>
<th>LF</th>
<th>LL</th>
<th>CHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Chinese</td>
<td>Chinese</td>
<td>Chinese</td>
</tr>
<tr>
<td>Consanguineous</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Family history</td>
<td>Younger brother (LL)</td>
<td>Older brother (LF)</td>
<td>No, only child</td>
</tr>
<tr>
<td>Gestation (weeks)</td>
<td>40</td>
<td>40</td>
<td>31</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>1.85 (IUGR)</td>
<td>2.0 (IUGR)</td>
<td>1.1</td>
</tr>
<tr>
<td>Current age (year)</td>
<td>8.5</td>
<td>2.8</td>
<td>11</td>
</tr>
<tr>
<td>Current weight-for-age</td>
<td>14.5 (severe FTT)</td>
<td>10.0</td>
<td>11.1</td>
</tr>
<tr>
<td>Intractable diarrhea</td>
<td>Yes</td>
<td>Intermittent diarrhea</td>
<td>Yes</td>
</tr>
<tr>
<td>Onset of diarrhea</td>
<td>Day 17</td>
<td>1 month</td>
<td>2 months</td>
</tr>
<tr>
<td>Typical facial appearance</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Wide forehead</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hypertelorism</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Trichorrhexis nodosa</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Jejunal villous atrophy</td>
<td>Yes</td>
<td>Not documented</td>
<td>Yes</td>
</tr>
<tr>
<td>Liver: hepatomegaly</td>
<td>No</td>
<td>Yes (2 cm)</td>
<td>Yes (6 cm)</td>
</tr>
<tr>
<td>Liver ultrasound</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Liver biochemistry</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Liver histology</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cardiac anomalies</td>
<td>Atrial septal defect, bicuspid aortic valve, not in failure</td>
<td>Bicuspid aortic valve, aortic stenosis, not in failure</td>
<td>Normal</td>
</tr>
<tr>
<td>Skeletal anomalies</td>
<td>Poor dentition</td>
<td>Poor dentition</td>
<td>Poor dentition</td>
</tr>
<tr>
<td>Renal anomalies</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Development</td>
<td>Delayed by ~1 year, short concentration span</td>
<td>Within normal limits</td>
<td>Within normal limits, home schooled</td>
</tr>
</tbody>
</table>

#### Nutrition

| Enteral nutrition | Yes | Yes | Yes |
| Parenteral nutrition | No | No | Yes (few years) |

#### Blood

| Thrombocytosis | Yes | Yes | No |
| Large platelets | No | No | No |

#### Liver biochemistry

- Serum immunoglobulins level
  - IgG (mg/dL, range 345–1236) 765
  - IgM (mg/dL, range 41–173) 321
  - IgA (mg/dL, range 14–159) 804
  - C3 (mg/dL, range 77–185) ND
  - C4 (mg/dL, range 7–40) ND

Liver biochemistry included total and conjugated bilirubins and liver enzymes (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl-transpeptidase).

* IUGR — intrauterine growth retardation; ND — not determined.
C4 were within normal limits for age (Table 1). The immunoglobulin IgG, IgA and IgM were also within normal limit (Table 1). The liver was just palpable. The ultrasound of the liver and serial monitoring of bilirubin and liver enzymes were all within normal limits. No liver biopsy was performed.

He received home parenteral nutrition for two years. However his parents decided to discontinue this after two years. Currently he is on normal diet with enteral supplements. He has severe FTT; the latest growth parameters were markedly below third percentile for age (Supplementary data). Developmentally, he is within normal limits and is receiving home schooling.

3.2. Molecular genetic studies

Family-1: The two affected siblings from the family-1 were found to have heterozygous mutations in the SKIV2L gene. The point mutations were located in exon-17 and exon-26 of the SKIV2L gene, respectively. No mutation was found in the TTC37 gene for both siblings. The mutation in exon-17 involves the substitution of guanine by adenine at position 1891 of the coding nucleotide (c.1891G>A; Fig. 3). This mutation involved the change of codon triplet from GGC to AGC and resulted in the replacement of glycine by serine in the amino acid chain at position 631 (p.Gly631Ser). Based on computational analysis, this substitution...
was predicted to be pathogenic (Polyphen-2: probably damaging — score: 1.00; SIFT: damaging — score: 0.00). On the other hand, the mutation in exon-26 replaced the cysteine with thymine at the position 3187 of the coding nucleotide (c.3187C>T; Fig. 3). The c.3187C>T mutation resulted in the switch of codon from CGA to TGA at position 1063 of the amino acid chain (p.Arg1063*). It caused the formation of a premature stop codon and thus a truncated protein with only 1062 amino acid residues, as opposed to 1246 amino acids in the normal peptide chain. Both of the point mutations in SKIV2L gene were absent in the ethnic-matched control group.

Family-2: No mutation was found in the proband in SKIV2L gene. One point mutation was observed in the TTC37 gene. The proband was homozygous for the point mutation in exon-33. The mutation in exon-33 involved a duplication of adenine after coding nucleotide at position 3426 (c.3426dupA; Fig. 4), this had directly led to amino acid change after position 1143 (p.Ala1143Serfs*4) due to a frameshift mutation. The mutant also formed a premature stop codon at position 1146. Mutation analysis results of the parents showed both parents are heterozygous carriers of the mutation in exon-33 in TTC37 gene. The point mutation was not observed in the ethnic-matched healthy controls.

4. Discussion

Based on the previous reports, 50% of the patients were born from consanguineous parents (Hartley et al., 2010; Fabre et al., 2013; Fabre et al., 2014). In the present study, we described three Malaysian Chinese children who had severe intrauterine growth retardation, early onset of severe and persistent diarrhea requiring parenteral or enteral nutrition, marked FTT, typical dysmorphic facies and trichorrhexis nodosa. These are the typical clinical features of THES (Hartley et al., 2010; Fabre et al., 2012). In the first family of this study, we had identified two point mutations in the SKIV2L gene in both affected siblings (c.1891G>A and c.3187C>T). Both were novel mutations that had not been described previously (Fabre et al., 2013). The c.1891G>A mutation is regarded as missense by the substitution of the amino acid at position 631. It was predicted to be highly pathogenic to the protein. The effect of a missense mutation can be expressed directly on the functionality of the resulted protein or by the alteration of its secondary structure and ability for protein–protein interactions, which may be associated to clinical manifestations in the affected individual (Davies et al., 2002; Yates and Sternberg, 2013). Unlike missense mutation, the nonsense mutation (c.3187C>T) is presumably devastating to the protein structure by introducing a premature stop codon. The transcription of a nonsense mutant produces a shorter and truncated protein that is usually not functional. The heterozygous C/T genotype of c.3187C>T

![Fig. 4. Sequencing results of TTC37 gene. (a) c.3426dupA in exon-33. (Upper left) Homozygous sample presents with a fifth adenine nucleotide (in box). (Upper right) Sample without the mutation contains only four adenines. (Lower) Reads from reverse primer for the father (S3F) and mother (S3M) of patient S3, the wavelike pattern occurred due to the presence of templates with varying length (+1 for the mutation and +0 for normal) in the heterozygous samples.]

Cases as reported previously showed that liver involvement was described in 50% of the patients with THES. In addition, of the 12 children with THES as described by Hartley et al. (2010), 9 of them required prolonged period of parenteral nutrition while 3 were dependent on parenteral nutrition. In comparison, two of the three patients as reported in this study were never required parenteral nutrition. Overall, milder phenotype observed in our three Malaysian patients did not due to their genotype influence as no genotype-phenotype correlation has been reported on 48 THES cases in a recent study (Fabre et al., 2013).

With times, the severity of diarrhea improved in some cases (Fabre et al., 2013). The major causes of mortality in children with THES were liver disease and recurrent infections (Fabre et al., 2013). Thus, although none of the three children reported in this study had any evidence of liver disease or recurrent infections, long term follow up is still necessary.

Mutations in patients with THES have always been associated with two genes: SKIV2L and TTC37 genes (Hartley et al., 2010; Fabre et al., 2012). A total of 9 mutations had been identified in SKIV2L gene while another 9 mutations had also been reported in TTC37 gene (Hartley et al., 2010; Fabre et al., 2012).
point mutation had been determined to present at an extremely low frequency, 0.001, by Exome Sequencing Project (ESP), with reference number of rs138923214 in GenBank. Therefore, the onset of THES in the siblings in family-1 was likely to be caused by the compound effect of both point mutations in heterozygous state.

For the second family, the change of amino acid due to frameshift mutation in TTC37 gene may result in the production of abnormal protein leading to a loss of binding groove or irregular protein structure. Furthermore, the formation of a premature stop codon following the point mutation triggers an early termination of the translation process, resulting in shorter and non-functional peptide chain. Therefore, the onset of THES in family-2 were heterozygous for the point mutation in TTC37 gene. They were phenotypically normal and did not have a history of persistent diarrhea, despite being the carrier for the mutation. This may be explained by the production of functionally normal thepisin by the wildtype strand.

Overall, incomplete of immunological investigation could be the major limitation of this study as qualitative B-cell function including immune response to immunization was not performed (Fabre et al., 2013). In addition, Sanger DNA sequencing approach used in the present study is only efficient for point mutation investigation, but not so sufficient for insertion and deletion detection.

THES has been well described in the literature, including a recent case report from Asia (Chong et al., 2015). It is likely that THES has a worldwide distribution, including Malaysia. The three cases observed in the present study should add on to the body of knowledge on what is known on THES in view of the milder phenotype observed.

5. Conclusion

In conclusion, we reported three novel mutations in the two causative genes (SKIV2L and TTC37) of THES in the Malaysian patients, including one nonsense, one frameshift, and one missense mutations. These mutations are predictive to be damaging by in silico analysis, perhaps through direct impact on the gene products by causing modification on the protein and impaired its functionality.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gene.2016.03.049.