Chinese patients with sporadic Hirschsprung’s disease are predominantly represented by a single RET haplotype

M-M Garcia-Barceló, M-H Sham, V C-H Lui, B L-S Chen, Y-Q Song, W-S Lee, S-K Yung, G Romeo, P K-H Tam

Hirschsprung’s disease is a developmental disorder characterised by the absence of ganglion cells in the nerve plexuses of the lower digestive tract. The Hirschsprung phenotype is variable and can be classified into two groups: SSA, or short segment aganglionosis, which includes patients with aganglionosis as far as the rectosigmoid junction; and LSA, or long segment aganglionosis, which includes patients with aganglionosis beyond the rectosigmoid junction. The condition presents in the neonatal period as a failure to pass meconium, chronic severe constipation, colonic distension, secondary electrolyte disturbances, and sometimes enterocolitis and bowel perforation.

The estimated population incidence is 1/5000 live births, although this is a representative value. The highest incidence is in Asian populations (2.8 per 10 000 life births) and the lowest in Hispanics (1 per 10 000 life births). The male to female (M:F) ratio is approximately 4:1 for SSA patients and 1:1 for LSA patients. Approximately 20% of cases are familial. The recurrence risks for siblings of SSA variant probands ranges between 1.5% and 3.3%, while the risks for those of LSA variant probands varies from 2.9% to 17.6%. Hirschsprung’s disease is often associated with chromosomal abnormalities, with other neurodevelopmental disorders such as Waardenburg syndrome type 4, and with a variety of additional isolated anomalies and syndromes.

The disease has a complex genetic aetiology, and many studies indicate the receptor tyrosine kinase gene (RET) as the major susceptibility gene for Hirschsprung’s disease. RET mutations are also associated with multiple endocrine neoplasia type 2 (MEN2), and medullary thyroid carcinoma. Papillary thyroid carcinoma is associated with RET somatic rearrangements.

Mutations in the RET gene account for up to 50% of the familial cases and anywhere between 7% and 35% of the sporadic cases, and they lack genotype-phenotype correlation. Other Hirschsprung genes identified so far mainly code for protein members of interrelated signalling pathways involved in the development of enteric ganglia: RET, endothelin receptor B (EDNRB), and the transcriptional regulator SOX10 signalling pathways. Mutations in the SIP1 gene have been found to cause syndromic Hirschsprung’s disease. These genes account for a small proportion of patients with this disorder (7%). Approximately 50% of affected individuals do not have mutations in the Hirschsprung genes. However, genetic linkage analyses in both LSA and SSA variant families repeatedly implicate the RET gene as a major susceptibility locus, even though the evidence of those linkage analyses is not reflected in the RET mutation status of many patients in whom RET coding region mutations were not found.

There are several possible explanations for the complex genetic aetiology of Hirschsprung’s disease:

- mutations in as yet unidentified genes;
- mutations in RET non-coding region that remain to be found;
- RET could be under epistatic regulation, and the interaction of several unlinked genes is required to produce the phenotype;
- RET specific SNPs, or a combination of these, could act as low susceptibility alleles or as factors modifying the phenotypic expression, or even be in linkage disequilibrium with an unknown susceptibility locus.

Key points

- Hirschsprung’s disease has a complex genetic aetiology, with RET being the major gene. This study was initiated to evaluate the RET haplotypes associated with the disease in the Chinese population. It was hypothesised that if the Hirschsprung phenotype is also related to the particular combinations of RET single nucleotide polymorphisms (SNPs), and if the incidence of those SNPs differs among populations, identification of those population specific alleles and haplotypes contributing to disease would help elucidate the molecular basis underlying Hirschsprung’s disease.
- The transmission disequilibrium test (TDT) and standard case–control statistics were used for comparison of RET haplotypes. Haplotypes were also compared between patients with different Hirschsprung’s disease phenotypes and RET mutation status.
- One main haplotype—allele A of c135G->A(A45A), allele G of c1296G->A(A432A), and allele G of c2307T->G(L769L)—represented 66% of the patients with Hirschsprung’s disease (A-G-G, x² = 22.23; p = 0.000002). Allele A of c1296G->A and haplotypes comprising it were associated with the more severe manifestations of the disease (p = 0.000074), regardless of the RET mutation status.
- The finding of A-G-G in 66% of Chinese patients with Hirschsprung’s disease indicates that they share the same genetic aetiology.
- These findings are relevant to Hirschsprung’s disease risk and to gene mapping, particularly for genome-wide linkage disequilibrium testing through the use of SNP markers.

Abbreviations: HWE, Hardy-Weinberg equilibrium; LSA, long segment aganglionosis; NT, chromosomes not transmitted; SNP, single nucleotide polymorphism; SSA, short segment aganglionosis; TDT, transmission disequilibrium test; TR, chromosomes transmitted
METHODS
Patients and controls
Eighty-seven ethnic Chinese patients diagnosed with sporadic Hirschsprung’s disease between January 1984 and April 2003 were included in the study. The diagnosis was based on histological examination of either biopsy or surgical resection material for the absence of enteric ganglia. Using polymerase chain reaction (PCR) amplification and direct sequencing, we screened the 21 exons of the RET gene, including intron/exon boundaries, from 87 patients, 52 available parents, and 100 ethnically matched controls. All individuals assented to the molecular analyses. The primers and PCR conditions used were as previously described. For exon 21, we generated the following pair of primers: 5'-AAAGGGAGTGTGCAAAAGCC-3' (forward) and 5'-TTAAAGTCTGAAAGGACGC-3' (reverse), which yielded a 157 base pair (bp) product.

Statistical analysis
The allele frequencies in patients and controls were calculated from the genotype frequencies and compared by the χ² test or Fisher’s exact test when an expected cell value was less than 5. Haplotype frequencies were estimated, using the EM algorithm as implemented in the EH program. EH is a less than 5. Haplotype frequencies were estimated, using the EM algorithm as implemented in the EH program. For each pair of loci, the two-locus haplotype frequencies were estimated and subsequently used for the analysis of haplotype association with disease risk. The statistical significance of the association was assessed using the χ² test with degrees of freedom (df) equal to the number of haplotypes minus 1.

RESULTS
RET mutations and polymorphisms
Sequencing analyses showed that 16 patients (18.4%) had potentially disease causing mutations in the RET gene (Garcia-Barceló M-M et al, unpublished data). Classification of the patients according to the length of the aganglionic segment (phenotype) and the RET mutation status is shown in Table 1.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>With mutation</th>
<th>Without mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSA (n = 73)</td>
<td>10 (62.5%)</td>
<td>63 (88.7%)</td>
</tr>
<tr>
<td>LSA (n = 14)</td>
<td>6 (37.5%)</td>
<td>8 (11.3%)</td>
</tr>
</tbody>
</table>

LSA, long segment agangliosis; SSA, short segment agangliosis.

Table 2: Allele frequencies of RET single nucleotide polymorphisms in 87 sporadic Chinese cases of Hirschsprung’s disease and 100 ethnically matched controls

<table>
<thead>
<tr>
<th>Exon</th>
<th>Nucleotide change</th>
<th>Codon</th>
<th>Controls (100)</th>
<th>Patients (87)</th>
<th>Statistic</th>
<th>Controls v patients</th>
<th>German v patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IVS1-17 T&gt;G (new)</td>
<td>–</td>
<td>0</td>
<td>1.1</td>
<td>χ²</td>
<td>1.15</td>
<td>0.28</td>
</tr>
<tr>
<td>2</td>
<td>c129 C&gt;G (new)</td>
<td>D43E</td>
<td>1</td>
<td>0</td>
<td>0.87</td>
<td>0.34</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>c135 G&gt;A</td>
<td>A45A</td>
<td>41</td>
<td>73</td>
<td>38.62</td>
<td>0.0001</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>c200 G&gt;A (new)</td>
<td>R67H</td>
<td>2.5</td>
<td>0.5</td>
<td>2.18</td>
<td>0.14</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>IVS2+9 G&gt;A</td>
<td>–</td>
<td>10.5</td>
<td>5.1</td>
<td>3.60</td>
<td>0.06</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>c375 C&gt;A</td>
<td>V125Y</td>
<td>0.5</td>
<td>0.5</td>
<td>0.01</td>
<td>0.92</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>c1294 G&gt;A</td>
<td>A432A</td>
<td>21</td>
<td>12</td>
<td>5.30</td>
<td>0.02</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>c1465 G&gt;A (new)</td>
<td>D489N</td>
<td>2</td>
<td>3.4</td>
<td>0.75</td>
<td>0.39</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>c2071 G&gt;A</td>
<td>G691S</td>
<td>10.5</td>
<td>4.5</td>
<td>4.53</td>
<td>0.03</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>c2037 C&gt;T (new)</td>
<td>P679P</td>
<td>0.5</td>
<td>0.5</td>
<td>0.01</td>
<td>0.92</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>c2307 T&gt;G</td>
<td>L76R</td>
<td>49</td>
<td>73</td>
<td>28.05</td>
<td>0.0001</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>c2508 C&gt;T</td>
<td>S836D</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>23.5</td>
</tr>
<tr>
<td>13</td>
<td>c2712 C&gt;G</td>
<td>S9045</td>
<td>10.5</td>
<td>4.5</td>
<td>4.53</td>
<td>0.03</td>
<td>ND</td>
</tr>
<tr>
<td>14</td>
<td>c2712 C&gt;G</td>
<td>S9045</td>
<td>10.5</td>
<td>4.5</td>
<td>4.53</td>
<td>0.03</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Fisher’s exact test; ND, not described.
Hirschsprung’s disease in our population were allele A of c135G>A (A45A), allele A of c1296G>A (A432A), allele G of c2307T>G (L769L), allele G of c2712C>G (S904S), and allele A of c2071G>A (G691S). Association of allele A of c135G>A (A45A), allele A of c1296G>A (A432A), and allele G of c2307T>G (L769L) was independently tested by TDT (table 3). The allele A of c1296G>A (A432A) was significantly underrepresented in Chinese patients.

**RET genotype distribution**

Forty two different genotypes encompassing the 13 RET SNPs found were observed among cases and controls (not shown). The overall genotype distribution differed significantly between cases and controls (χ² = 242.46; p = 0.000001). One to one genotype comparisons showed that four main genotypes of (42) showed a significant difference between patients and controls (genotypes 1 to 4 in table 4). The most common disease associated RET genotype (50.6%; genotype 1) was that homozygous for both allele A of c135G>A (A45A) and allele G of c2307T>G (L769L), and wild-type for the rest of polymorphisms found. Genotypes heterozygous for these two polymorphisms (genotypes 2 and 8) were instead overrepresented in the control population.

**Identification of the main RET haplotype in Chinese patients with Hirschsprung’s disease**

We constructed the haplotypes comprising the three most significantly associated RET SNPs in our population (c135G>A (A45A), c1296G>A (A432A), and c2307T>G (L769L)) and on the basis of published reports. Significant differences were observed in the global haplotype distribution between patients and controls (χ² = 42.46; p<0.00001 for haplotypes; χ² = 57.52; p<0.00001 for genotypes). One to one comparison revealed significant differences between patients and controls for haplotypes A-G-G, G-G-T, and G-A-T. A TDT test done in 52 parent–proband trios confirmed the results (table 5). A-G-G was the most common haplotype in our population, with an estimated frequency of 66%.

**RET-SNPs and RET haplotypes in different Hirschsprung phenotypes**

To investigate whether RET-SNPs are associated with a particular Hirschsprung phenotype, we compared the allelic distribution of the polymorphisms described above between patients with long/totall segment aganglionosis (LSA) and patients with short segment aganglionosis (SSA), and also between patients harbouring mutations in RET or not. Only allele A of c1296G>A (A432A) was significantly under-represented in SSA patients (table 6). TDT, done in 45 parent–proband trios with SSA, showed that allele G was the most frequently transmitted allele (TR:NT 22:6; χ² = 9.14; p = 0.002). None of the SNPs revealed statistically significant differences in frequencies between patients with RET mutation and those without (table 6).

**Three-loci haplotype**

To test the previously observed association of allele A of c1296G>A (A432A) with LSA, we investigated whether the haplotype distribution differed between LSA and SSA patients. Allele A had already been found significantly under-represented when all patients with Hirschsprung’s disease were compared with controls, and from these observations a protective role for this allele seemed plausible. However, the frequency of allele “A” in patients affected with LSA, although not statistically significant, was higher than for the same allele in controls, which is at odds with a
protective role. This suggested that the association of allele A with LSA was in combination with other polymorphisms within RET.

Global haplotype and genotype distributions showed statistically significant differences between SSA and LSA patients ($\chi^2 = 20.84; p = 0.004$ for haplotypes, and $\chi^2 = 35.9; p = 0.0006$ for genotypes). Haplotype A-A-G was more common in LSA patients (14% vs 0% in SSA; Fisher’s two tailed exact test, $p = 0.000075$) and haplotype A-G-G in SSA (69.7% vs 46.4% in LSA; $\chi^2 = 16.3; p = 0.00041$). TDT confirmed the preferential transmission of the haplotype A-G-G in the SSA group (table 7).

Although parents were only available for seven LSA patients, TDT results showed a statistically significant trend towards the transmission of A-G-G in this group. It is likely that the TDT values did not reach statistical significance in LSA patients owing to the small sample size analysed. However, as our patients and controls are ethnically matched, there is little distortion of the data obtained by EH, and the significance reached by case-control standard statistics accurately reflects the trends observed.

The results obtained in this three-loci haplotype analysis suggest that while both allele A of c135G>A (A45A) and allele G of c2307T>G (L769L) are associated with Hirschsprung’s disease independently of the severity of the phenotype, allele A of c1296G>A (A432A) in combination with the variant alleles of c135G>A (A45A) and c2307T>G (L769L) is associated with the more severe form of Hirschsprung’s disease.

### Two-loci haplotype

To determine whether the association found for c1296G>A (A432A) was driven by the other polymorphic alleles associated with Hirschsprung’s disease, three two-loci haplotype distributions—c135G>A (A45A)-c2307T>G (L769L), c135G>A (A45A)-c1296G>A (A432A), and c1296G>A (A432A)-c2307T>G (L769L)—were compared between LSA and SSA patients. As expected, for the combination c135G>A (A45A)-c2307T>G (L769L), no difference between SSA and LSA patients was found. However, significant differences in the global genotype and haplotype distributions between LSA and SSA patients were observed for the combination (c135G>A (A45A)-c1296G>A (A432A) ($\chi^2 = 19; p = 0.0012$ for genotypes and $\chi^2 = 12.64; p = 0.0054$ for haplotypes), and the combination c1296G>A (A432A)-c2307T>G (L769L) ($\chi^2 = 18.9; p = 0.001$ for genotypes, and $\chi^2 = 13.08; p = 0.0004$ for haplotypes). In particular, for c135G>A (A45A)-c1296G>A (A432A), haplotype A-G was predominant in SSA patients (73% vs 49% in LSA; $\chi^2 = 12.11; p = 0.0005$) and this was confirmed by TDT
done in 45 SSA trios (TR:NT 38.8; \( \chi^2 = 19.57; p = 0.00001 \)). Haplotypes A-A was significantly more common in LSA patients (18% vs 0.8% in SSA; \( \chi^2 = 17.37; p = 0.000031 \)).

As for pair c1296G>A(A432A)-c2307T>G(L769L), the most dramatic difference between SSA and LSA was that of the haplotype A-G (1.5% in SSA vs 24% in LSA; \( \chi^2 = 22.8; p = 0.000002 \)). Haplotypes G-G was significantly overrepresented in the SSA group (73% vs 53%; \( \chi^2 = 8.6; p = 0.003 \)). TDT done in the SSA group also showed that G-G was preferably transmitted (TR:NT 38:9; \( \chi^2 = 17.89; p = 0.000002 \)).

Two-loci haplotype analysis confirmed that allele A of c1296G>A(A432A) in cis combination with either allele A of c135G>A(A45A) or allele G of c2307T>G(L769L) is associated with LSA.

Two- and three-loci haplotype distributions showed no differences between patients with RET mutation and patients without (data not shown).

**RET-SNPs and RET haplotypes in other populations**

Except for allele A of c1296G>A(A432A), those polymorphic alleles associated with Hirschsprung’s disease in Chinese patients (in particular allele A of c135G>A(A45A) and allele G of c2307T>G(L769L)) are also associated with the disease in white patients. However, the **RET-SNP** frequencies found in our population (in both patients and controls) differ considerably from those reported for the same SNPs in white subjects (table 2). For instance, the frequency of allele A of c135G>A(A45A) varied greatly between Chinese and white control populations (41% vs 16–23%, respectively) but remained rather similar for the two patient populations (73% in Chinese vs 59–73.4% in whites). Allele G of c2307T>G(L769L) occurred much more often in Chinese patients (75.2%) than in white patients (30.5%–42.7%). In addition, while the frequencies of the variant alleles of c135G>A(A45A) and c2307T>G(L769L) were similar in the Chinese population (patients and controls), they differed greatly in white subjects (table 2). As to the c2508C>T(S836S) polymorphism, the variant allele T was not found either in patients or controls. In whites, c2508C>T(S836S) is characterised by the low frequency of the allele T in patients with Hirschsprung’s disease and its overrepresentation in medullary thyroid carcinoma.33–40

The ethnic differences observed in the SNP frequencies were reflected in the frequencies of the **RET** genotypes/haplotypes associated with Hirschsprung’s disease. Genotypes and haplotypes encompassing the same SNPs were compared with those previously reported for the **RET** haplotype study done in white (Spanish) patients with Hirschsprung’s disease (table 4).41 While 50.6% of the Chinese patients shared the same genotype—haplotype 1; homozygous for both allele A of c135G>A(A45A) and allele G of c2307T>G(L769L) and wild-type for the rest of the polymorphisms found—five different genotypes (BB, BC, BD, BH and CD) together accounted for a similar number (48%) of Spanish patients. In addition, those four genotypes were rare in our patient population—that is, the most common Hirschsprung’s disease genotype in Spanish people (BB) is not observed in the Chinese. Furthermore, the most common Hirschsprung haplotype seen in Chinese people (A-G-G; 66%; c135G>A(A45A), c1296G>A(A432A), c2307T>G(L769L); table 5) was only seen in 19% of the Spanish patients; vice versa, the most common Hirschsprung haplotype in Spaniards (30.7%; haplotype B: allele A of c135G>A(A45A) and wild-type for the other sites) was only seen in 3.7% of the Chinese patients. Genotypes and haplotypes comprising both allele A of c135G>A(A45A) and allele G of c2307T>G(L769) are particularly associated with Hirschsprung’s disease in Chinese people, while in whites the main genotype and haplotype associated with Hirschsprung’s disease comprise only allele A of c135G>A(A45A).

**DISCUSSION**

The current data on Hirschsprung’s disease indicate that **RET** haplotypes associated with this condition can themselves be functional and lead to a low penetrance loss of function, act as modifiers of the phenotype conferring risk/protection to disease (additive/combinatorial effect of multiple **RET** polymorphisms, combinatorial hypothesis), or represent linkage disequilibrium with a low penetrance susceptibility locus. We initiated this study to evaluate the **RET** “risk” haplotypes present in our population, to elucidate the molecular basis of Hirschsprung’s disease. We hypothesised that if the Hirschsprung’s disease phenotype is related to the particular combinations of **RET**-SNPs, and if the incidence of those polymorphic variants differs among populations, identification of those population specific SNPs and haplotypes contributing to disease would definitely help in the discovery of the molecular basis underlying Hirschsprung’s disease.

Our data showed that Chinese patients with Hirschsprung’s disease are mainly represented by one **RET** haplotype (comprising variant alleles of c135G>A(A45A) and c2307T>G(L769L)), indicating that if this haplotype was itself functional, 66% of the Chinese patients would share the same genetic aetiology. The existing data on white patients with Hirschsprung’s disease show that the haplotype distribution is more diverse and that c135G>A(A45A) alone is driving a strong association between haplotypes containing this polymorphism (allele A) and Hirschsprung’s disease. Those differences between populations in the frequencies of the **RET** haplotypes with the best evidence for an association with Hirschsprung’s disease suggest that the true **RET** disease susceptibility allele remains to be identified; alternatively they might suggest that different haplotypes in different populations confer risk of or protection against disease, and those “functional haplotypes” would also account for the patients with Hirschsprung’s disease with no mutations in any of the Hirschsprung genes.

In favour of the first explanation, it has been suggested recently that in Spaniards,33 **RET** “risk” haplotypes appeared to be in linkage disequilibrium with a putative founding low penetrance susceptibility locus, located upstream of c135G>A(A45A). Thus it could be that the “true” susceptibility locus is on different haplotype backgrounds because of human population and history structure, explaining the differences found in the **RET** haplotypes associated with Hirschsprung’s disease. The main **RET** haplotype associated with Hirschsprung’s disease in Chinese people still belongs to the group of haplotypes in linkage disequilibrium with the low penetrance susceptibility locus upstream of A45A, as previously described in whites.35 The differences in “risk haplotype” frequencies between Chinese and whites could indicate that recombination events within the region flanked by A45A and L769L may have occurred (after the European–Asian split). This may have originated in two “risk haplotypes,’’ one mainly represented by allele A of A45A, more common in Europeans, and another, more common in the Chinese, represented by the variants alleles of A45A and L769L. Both haplotypes could be in linkage disequilibrium with the low susceptibility locus upstream A45A previously described.31

The second explanation is not mutually exclusive with the presence of a putative locus in linkage disequilibrium with **RET** “risk” haplotypes, because particular combinations of SNPs on those haplotypes could also act as modifiers.30–40

We also found that allele and haplotype distributions varied with the severity of the Hirschsprung phenotype: allele A of c1296G>A(A432) in combination with the variant...
alleles of c135G>A(A45A) and c2307T>G(L769L) was associated with the more severe form of Hirschsprung’s disease, while both c135G>A(A45A) and c2307T>G(L769L) polymorphisms were associated with Hirschsprung’s disease independently of the severity of the phenotype. However, these data should be viewed with caution until further proof is provided by functional analysis.

Differences in allele frequencies between different Hirschsprung phenotypes have never been reported for c1296G>A(A432A). Instead, in a study of 76 white patients from Germany, differences in the frequency of allele A of c135G>A(A45A) were found between SAS and LSA patients, and also between patients with the RET mutation and those without. It was also shown that c135G>A(A45A) polymorphism can modify the phenotype through a within-gene interaction. In our series c135G>A(A45A) was not associated with any Hirschsprung phenotype or with RET mutations. Instead, c1296G>A(A432A) and haplotypes comprising it were associated with the more severe form of the disease. No differences were found in allele and haplotype distributions when patients harbouring or not harbouring the RET mutation were compared, which could indicate that RET specific haplotypes may influence the Hirschsprung’s disease phenotype regardless of the RET mutation status. This influence could be through interaction of the RET-SNPs within RET itself or with other genes.

RET haplotypes containing the allele T of c2508C>T(S836S) have a protective role in the pathogenesis of Hirschsprung’s disease. This allele has not been found in the Chinese, so there is ethnic variation in the polymorphisms implicated in the phenotype modulation of Hirschsprung’s disease and possibly mediulary thyroid carcinoma. Interestingly, differences in the distribution of the RET haplotypes have also been detected among patients with papillary thyroid carcinoma from different white populations.

Transmission of mutations in other Hirschsprung genes (EDNRB, GDNF) has been found to be associated with the transmission of RET polymorphisms, proving the genetic interaction underlying Hirschsprung’s disease. Combinations of variants in several components of the RET and EDNRB signalling pathways might overall provide an important risk factor for disease susceptibility and also contribute to the necessary response to produce the Hirschsprung phenotype. When a candidate gene contains various polymorphisms, these are usually in linkage disequilibrium because the relatively small genetic distances make it difficult in a single population to dissect out the important polymorphism. Ethnic differences in the haplotypes associated with disease can make it easier to isolate the SNP that actually confers susceptibility to a given disease at a given locus. Thus our data are highly relevant to Hirschsprung’s disease risk and to gene mapping, particularly for genome-wide testing for linkage disequilibrium through the use of SNP markers.

ACKNOWLEDGEMENTS
We extend our gratitude to all subjects who participated in the study. We thank Professor Pak Shum for critical review of the manuscript and helpful discussions. This work was supported by research grants from the Hong Kong Research Grants Council (HKU 73580/M01).


