Heterozygous endothelin receptor B (EDNRB) mutations in isolated Hirschsprung disease

Jeanne Amiel, Tania Attié, Dominique Jan, Anna Pelet, Patrick Edery, Christelle Bidaud, Didier Lacombe¹, Paul Tam², Juliette Simeoni³, Elisabeth Flori⁴, Claire Nihoul-Fékété, Arnold Munnich and Stanislas Lyonnet*

Unité de Recherches sur les Handicaps Génétiques de l’Enfant INSERM U-393, Service de Génétique Médicale and Clinique Chirurgicale Infantile, Hôpital des Enfants Malades, 149, rue de Sèvres, 75743, Paris Cedex 15, France, ¹Service de Génétique Médicale, CHRU de Bordeaux, 33076 Bordeaux Cedex, France, ²Paediatric Surgery, John Radcliffe Hospital, Oxford, UK, ³Service de Chirurgie, Hôpital d’Enfants de la Timone, 13385 Marseille Cedex, France and ⁴Laboratoire de Cytogénétique, Hôpital de Hautepierre, 67098 Strasbourg Cedex, France

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Hirschsprung disease (HSCR, aganglionic megacolon) is a frequent congenital malformation regarded as a multigenic neurocristopathy. Two susceptibility genes have been recently identified in HSCR, namely the RET proto-oncogene and the endothelin B receptor (EDNRB) gene. Hitherto however, homozygosity for EDNRB mutations accounted for the HSCR-Waardenburg syndrome (WS) association. Here, we report heterozygous EDNRB missense mutations (G57S, R319W and P383L) in isolated HSCR. These data might suggest that EDNRB mutations could be dosage sensitive: heterozygosity would predispose to isolated HSCR with incomplete penetrance, while homozygosity would result in more complex neurocristopathies associating HSCR and WS features. In addition, the present data give further support to the role of the endothelin-signalling pathway in the development of neural crest-derived enteric neurons.

INTRODUCTION

Hirschsprung disease (HSCR, aganglionic megacolon) is a frequent congenital malformation (1/5000 live births) resulting in intestinal obstruction in neonates and severe constipation in infants and adults (1–2). HSCR is characterized by the absence of parasympathetic intrinsic ganglion cells in the submucosal and myenteric plexuses of the hindgut (3). HSCR has been divided into short-segment (80%) and long-segment forms (20%), based on length of the aganglionic tract. The disease has long been regarded as a multigenic condition, and segregation analyses supported an autosomal dominant mode of inheritance with incomplete penetrance in long-segment HSCR, while the autosomal recessive or multifactorial inheritance were equally likely in short-segment HSCR (4). The RET proto-oncogene has been recognized as a major gene in autosomal dominant HSCR (5–8). Yet, RET mutations only account for 50% and 15–20% of familial and sporadic cases, respectively, supporting the involvement of other susceptibility gene(s) in HSCR (9). Recently, homozygous mutations of the endothelin B receptor (EDNRB) gene have been identified in consanguinous HSCR families harboring other malformations of neural crest-derived cells, namely pigmentary anomalies and deafness (Shah-Waardenburg syndrome; refs 10,11). However, the question of whether EDNRB mutations also account for isolated HSCR remained unanswered. Here, we report heterozygous deletions and EDNRB missense mutations in seven HSCR patients. These data support the involvement of EDNRB mutations in isolated HSCR and emphasize the role of the endothelin-signalling pathways in the development of the enteric nervous system.

RESULTS

Abnormal SSCP patterns were detected in 4/165 probands. In 3/165 cases, heterozygous variations in exons 1, 5 and 6 predicted missense mutations of the extracellular (G57S), the third intracellular (R319W) and the seventh transmembrane domain (P383L) of the EDNRB protein respectively (Fig. 1 and Table 1). In one case, a heterozygous G to A transversion located in the 5' untranslated region (5' UTR) of the gene was observed. In each of the four cases, the mutation was found to be inherited from an asymptomatic carrier (Table 1). The mutations were absent in 65 unrelated healthy individuals (130 chromosomes). The rest of the coding sequence was similar to controls and no abnormal SSCP patterns were detected in the RET gene and in the EDNRB ligand coding gene, endothelin 3 (EDN3, data not shown). Finally, the parental origin of the deletion could be identified in the three HSCR patients harboring chromosome 13q22 deletions (two paternal, one maternal) and in two of them, large scale deletions encompassing the EDNRB gene could be documented using flanking polymorphic markers (Fig. 2).

*To whom correspondence should be addressed
Table 1. EDNRB gene point mutations in patients with isolated HSCR

<table>
<thead>
<tr>
<th>Patients (sex)</th>
<th>Exon</th>
<th>Nucleotide change</th>
<th>Mutation</th>
<th>Origin of mutation</th>
<th>Familiality of HSCR</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>S133 (M)</td>
<td>E1</td>
<td>–26 G→A</td>
<td>5' UTR</td>
<td>paternal</td>
<td>sporadic</td>
<td>short-segment</td>
</tr>
<tr>
<td>S82 (F)</td>
<td>E1</td>
<td>GGT→A*GT</td>
<td>G57S</td>
<td>maternal</td>
<td>sporadic</td>
<td>short-segment+ NID</td>
</tr>
<tr>
<td>S140 (M)</td>
<td>E5</td>
<td>GGG→T*GG</td>
<td>R319W</td>
<td>maternal</td>
<td>sporadic</td>
<td>short-segment</td>
</tr>
<tr>
<td>S137 (F)</td>
<td>E6</td>
<td>CCA→CT*A</td>
<td>P383L</td>
<td>maternal</td>
<td>familial*</td>
<td>short-segment</td>
</tr>
</tbody>
</table>

The first nucleotide of the traduction initiation codon was numbered as +1. M: male, F: female, NID: neuronal intestinal dysplasia. *A second degree relative was affected with short-segment HSCR.

**DISCUSSION**

The EDNRB gene encodes a 442 amino acid heptahelical receptor that equally binds EDN 1, 2 and 3, and is involved in the intracellular signalling pathway via heterotrimeric G proteins (12,13). The missense mutations reported here occurred in highly conserved regions of the protein. Indeed, the third cytoplasmic loop which encompasses the R319W mutation (Fig. 1) is critical for G-protein coupling, while ligand binding requires integrity of the transmembrane domain where the P383L mutation takes place (14). Yet, the responsibility of the 5' UTR mutation (–26 G→A) in the disease remains questionable. Finally, large deletions encompassing the EDNRB gene might suggest haplo-insufficiency as the cause of HSCR in chromosome 13q22 deletions, especially as no mutation of the non-deleted EDNRB allele could be identified by direct sequencing.

Interestingly, the heterozygous EDNRB mutations reported here were consistently inherited from an unaffected carrier. This feature gives support to the low penetrance of the trait and is in agreement with the observation that the W276C EDNRB mutation was neither necessary nor sufficient to produce the HSCR phenotype in the Mennonite kindred (10). Taken together, these data support the existence of one or more modifier loci (different from RET and EDN 3) in HSCR individuals carrying heterozygote EDNRB mutations.

It is important to note that homozygous disruption of the EDNRB or EDN3 genes in mice resulted in megacolon and white coat-spotting, as observed in the piebald lethal and lethal spotting natural mutants respectively (15,16). Accordingly, homozygous EDNRB or EDN3 mutations predisposed to pigmented anomalies and deafness in HSCR patients (10,11,17) while heterozygous EDNRB mutations caused HSCR with no associated features. EDNRB could be regarded therefore as a susceptibility locus in non-syndromic HSCR (4/165 probands in our series). In conclusion, previous reports (10,11) together with the data presented here suggest that EDNRB mutations in human could be dosage sensitive; heterozygosity would predispose to isolated HSCR with incomplete penetrance, while homozygosity would result in more complex neurocristopathies associating HSCR and features of the Waardenburg syndrome. The question of whether modifying alleles at the EDNRB locus could also account for the low penetrance of RET mutations in HSCR families is now open to debate.

**MATERIALS AND METHODS**

A total of 165 isolated HSCR probands (85 sporadic, 80 familial) were tested for mutations in the coding sequence of the EDNRB gene. The first nucleotide of the traduction initiation codon was numbered as +1. M: male, F: female, NID: neuronal intestinal dysplasia. *A second degree relative was affected with short-segment HSCR.
Results

gene (seven exons). Histopathological criteria for HSCR were absence of enteric plexuses with histological evaluation of the aganglionic tract and increased acetylcholinesterase histochemistry in nerve fibres. Three short-segment HSCR patients with cytogenetically visible deletions of the 13q22 band encompassing the EDNRB locus with respect to physical map of the 13q22 region (10). Thick line: 2 copies, dotted lines: non-informative. Patients S190 and A have been previously reported by Lamont and coworkers (Cases 1 and 2 respectively, ref. 18).

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REFERENCES


Figure 2. Large scale deletions encompassing the EDNRB locus with respect to physical map of the 13q22 region (10). Thick line: 2 copies, dotted lines: non-informative. Patients S190 and A have been previously reported by Lamont and coworkers (Cases 1 and 2 respectively, ref. 18).