Exclusion of RET and Pax 3 loci in Waardenburg-Hirschsprung disease

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Abstract

The RET and the Pax 3 genes have recently been shown to account for autosomal dominant Hirschsprung’s disease (HSCR) and Waardenburg syndrome type 1 (WS1) respectively, which led us to consider them as candidate genes in the WS/HSCR association. Linkage analyses performed in a consanguineous WS/HSCR family support the view that neither the RET locus nor the Pax 3 locus are involved in the disease phenotype. Hence, at least one further locus altering neural crest cell development is responsible for the pleiotropic features observed in the WS/HSCR association.

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Hirschsprung’s disease (HSCR) is a common congenital disorder (1/5000 live births) ascribed to the absence of parasympathetic intrinsic ganglion cells in the submucosal and myenteric plexus of the hindgut. HSCR results in intestinal obstruction in neonates and infants and severe constipation in adult patients. We have recently mapped a dominant gene for familial HSCR to the proximal long arm of chromosome 10 (10q11.2), in close proximity to the RET proto-oncogene locus. Shortly thereafter, we and others identified nonsense and missense mutations of the RET gene in HSCR patients. Waardenburg syndrome (WS, MIM 193500) is an autosomal dominant malformation syndrome (1/50 000 live births), characterised by congenital deafness, dysmorphic features, and pigmentary anomalies of the hair, irides, and skin. Two types of WS have been recognised based on the presence (WS1) or absence of dystopia canthorum (WS2). A gene for WS1 has been mapped to the distal long arm of chromosome 2 (2q35-q37) and mutations of the gene Pax 3 have subsequently been identified that account for the majority of WS1 patients.

HSCR and WS are regarded as neurocristopathies related to abnormal migration of neural crest cells towards either the enteric nervous system (HSCR) or the skin and craniofacial fields (WS). The WS/HSCR association has been previously reported (Shah-Waardenburg syndrome or WS4, MIM 277580) and is regarded as an inherited condition, although its mode of inheritance remains debatable. The recent identification of genes for HSCR and WS1 led us to consider RET and Pax 3 as candidate genes in the WS/HSCR association. Here, we report the results of linkage analyses in a multiplex consanguineous family with WS/HSCR and show that the disease could not be accounted for by mutations in either the RET or the Pax 3 genes. These data suggest that an additional gene altering neural crest cell development is responsible for the pleiotropic features observed in the WS/HSCR association.

Family report

Diagnostic criteria for WS included at least two of the following traits: deafness, dystopia canthorum, heterochromia irides, and white forelock. Histopathological criteria for HSCR were absence of enteric ganglia cells and increased acetylcholinesterase histochemical staining in amyelinic nerve fibres. A consanguineous WS/HSCR family (first cousin parents) with two affected children was analysed (table). The proband was the third child of Tunisian parents (II-3, figure). Abdominal distension and faecal vomiting at 13 days of life led to the diagnosis of rectosigmoidal HSCR. Heterochromia irides and a white forelock were noted but no dystopia canthorum, telecanthus, or white spots on the skin were present. At 1 year of age, complete sensorineural deafness was diagnosed. Her sister (II-4, figure) presented at birth with intestinal occlusion requiring ileostomy. Rectosigmoidal and colonic biopsies provided evidence of aganglionicism, and long segment HSCR was diagnosed. She also had a white forelock, hypochromia irides, white skin spots on the right upper limb, and total sensorineural deafness, but no dystopia canthorum (WS2). The parents were healthy and the two older brothers had no intestinal involvement, hearing loss, or cutaneous abnormalities.

Methods and results

The HSCR/WS family was genotyped using microsatellite DNA markers closely flanking the RET locus (probes STCL-2 and D10S141) or located within the Pax 3 gene (Pax 3 probe). The WS/HSCR association was tested as either

Clinical data on WS/HSCR family. Subjects are designated by their predigree number (figure).

<table>
<thead>
<tr>
<th>Patient</th>
<th>II-3</th>
<th>II-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deafness</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dystopia canthorum</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>White forelock</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Skin pigmentation disorder</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Heterochromia irides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hirschsprung’s disease</td>
<td>Rectosigmoidal</td>
<td>Colonic</td>
</tr>
</tbody>
</table>
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Pedigree of \( \text{sTCL2} \) and \( \text{D10S141} \) loci in the WS/HSCR family. Family members are presented with their genotypes at \( \text{D10S141} \), \( \text{sTCL2} \) (chromosome 10q11.2), and \( \text{Pax} \, 3 \) (chromosome 2q35) loci.

Pax 3 locus in the mouse. Once these genes have been cloned, they should be regarded as strong candidate genes in the WS/HSCR association in man.

In conclusion, the data presented here support the view that familiar HSCR, WS, and WS/HSCR are distinct genetic entities and at least one additional locus altering neural crest cell development is responsible for the pleiotropic features observed in the WS/HSCR association.

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8 Of the two affected sibs shared common haplotypes with the unaffected sibs at this locus (II-1 and II-2 respectively).

Discussion

We report here a new WS/HSCR family and show that neither RET nor \( \text{Pax} \, 3 \) accounted for the WS/HSCR association. Convincing evidence for the exclusion of the two loci was based on the comparison between the affected sibs. Moreover, both dominant and recessive inheritance was ruled out, as the affected sib had no shared haplotype at either locus. Therefore, it is likely that another gene (or genes) is (are) responsible for the WS/HSCR association in the family studied.

Of the five WS/HSCR families reported by Badner and Chakravarti, two were consanguineous. The consanguineous family reported here reinforces the view that the WS/HSCR association may follow an autosomal recessive mode of inheritance. These data contradict previous segregation analyses and further studies are required to determine whether a dominant or a recessive mode of inheritance is involved in WS/HSCR families.

No dystopia canthorum has been present in WS/HSCR patients hitherto reported. These features should prompt one to look carefully for minor signs of WS in HSCR patients and related subjects, regardless of the length of the aganglionic segment.

Aganglionosis and pigmented disorders have been described in several mouse strains, or namely the piebald-lethal (s), lethal spotted (l), and Dominant spotting (Dom), but none of these mutant genes maps to the RET locus or to the