Genes Associated with Isolated HSCR

Genes for RET and its ligands. The tyrosine kinase receptor, RET (proto-oncogene tyrosine-protein kinase receptor; rearranged during transfection), is expressed by enteric neural precursors shortly after they leave the neural plate and throughout their colonization of the entire gut. GDNF (glial cell line-derived neurotrophic factor) and NRTN (or NTN; neurturin) are two of the ligands for RET expressed by adjacent mesenchymal cells. Although coreceptors for RET and its ligands exist, screening for variants in the specific coreceptor associated with GDNF (GFR alpha-1) has not revealed any pathogenic variants in humans [Myers et al 1999].

Pathogenic variants in RET appear to be dominant loss-of-function variants with reduced penetrance and variable expressivity. RET pathogenic variants alone are estimated to account for 7%-41% of all individuals with HSCR and 70%-80% of those with long-segment disease [Angrist et al 1995, Seri et al 1997, Sancandi et al 2000]. Homozygous RET pathogenic variants have been associated with total colonic aganglionosis in some individuals [Inoue et al 2000, Shimotake et al 2001].

RET is implicated in up to 50% of all familial HSCR [Attie et al 1995, Hofstra et al 2000] and in 10%-35% of simplex cases of HSCR (i.e., HSCR in a single family member) in several referral series, [Angrist et al 1995, Attie et al 1995, Eng & Mulligan 1997].

Common benign variants in RET that do not cause amino acid changes are over-represented in individuals with HSCR [Borrego et al 1999, Fitze et al 2003, Borrego et al 2000, Emison et al 2010], reflecting the complex nature of the disorder and the challenge of determining if a sequence variant is pathogenic.

- One common RET benign variant, rs2435357 C>T, is found in 79% of individuals with HSCR in both European and Asian populations (see table). This benign variant localizes to the first intron of RET and the HSCR-associated benign variant abrogates binding of the SOX10 at a gastrointestinal-specific enhancer, thereby reducing RET expression [Garcia-Barcelo et al 2005, Emison et al 2010]. This benign variant is associated with male gender, short- or long-segment HSCR (but not total colonic aganglionosis), and simplex cases [Kapoor et al 2015].

- Another RET benign variant rs2506030 A>G is found ~125 kb upstream of the gene and also confers increased susceptibility to developing HSCR in individuals of European ancestry, but does not show the same genotype-phenotype correlations as rs2435357 [Kapoor et al 2015].
In addition to the differential effects in males and females harboring the rs2435357 \textit{RET} benign variant [Emison et al 2005, Emison et al 2010], the variation in penetrance of HSCR between males and females may also be related to the role of the SRY (sex-determining gene on the Y chromosome) protein in regulating the expression of \textit{RET} by competitively binding to its transcriptional enhancer elements [Li et al 2015].

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP ID and risk/ non-risk allele</th>
<th>Case-control (risk allele frequency)</th>
<th>Odds ratio (95% CI)</th>
<th>P</th>
<th>TDT (risk allele transmitted/untransmitted) (T/U)</th>
<th>Odds ratio (95% CI)</th>
<th>P</th>
<th>Transmission rate (t ± sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RET</td>
<td>rs2435357: T/C</td>
<td>0.58/0.26</td>
<td>3.9 (3.2–4.7)</td>
<td>4.3 × 10^{-4}</td>
<td>219/50</td>
<td>4.4 (3.2–6.0)</td>
<td>6.8 × 10^{-25}</td>
<td>0.82 ± 0.02</td>
</tr>
<tr>
<td>RET</td>
<td>rs2506030: G/A</td>
<td>0.56/0.41</td>
<td>1.8 (1.5–2.2)</td>
<td>4.7 × 10^{-10}</td>
<td>164/93</td>
<td>1.8 (1.4–2.3)</td>
<td>9.5 × 10^{-6}</td>
<td>0.63 ± 0.03</td>
</tr>
<tr>
<td>SEMA3</td>
<td>rs11766001: C/A</td>
<td>0.22/0.15</td>
<td>1.5 (1.3–2.0)</td>
<td>1.0 × 10^{-4}</td>
<td>96/55</td>
<td>1.7 (1.3–2.1)</td>
<td>8.5 × 10^{-4}</td>
<td>0.64 ± 0.04</td>
</tr>
<tr>
<td>SEMA3</td>
<td>rs12070682: C/T</td>
<td>0.30/0.24</td>
<td>1.3 (1.1–1.6)</td>
<td>0.01</td>
<td>114/92</td>
<td>1.2 (0.9–1.6)</td>
<td>0.13</td>
<td>0.57 ± 0.03</td>
</tr>
<tr>
<td>SEMA3</td>
<td>rs1583147: T/C</td>
<td>0.28/0.23</td>
<td>1.3 (1.1–1.6)</td>
<td>0.01</td>
<td>115/86</td>
<td>1.3 (1.0–1.8)</td>
<td>0.04</td>
<td>0.57 ± 0.03</td>
</tr>
<tr>
<td>NRG1</td>
<td>rs16879552: C/T</td>
<td>0.07/0.96</td>
<td>1.2 (0.7–2.1)</td>
<td>0.43</td>
<td>13/15</td>
<td>0.9 (0.4–1.8)</td>
<td>0.71</td>
<td>0.50 ± 0.09</td>
</tr>
<tr>
<td>NRG1</td>
<td>rs7835668: G/G</td>
<td>0.49/0.47</td>
<td>1.1 (0.9–1.3)</td>
<td>0.44</td>
<td>134/124</td>
<td>1.1 (0.8–1.4)</td>
<td>0.53</td>
<td>0.53 ± 0.03</td>
</tr>
</tbody>
</table>

For case-control association, the risk allele frequency in cases and controls, odds ratio with 95% confidence interval (CI) and the significance value of association (P) are provided. For TDT, the counts of risk allele transmitted and un-transmitted from heterozygous parents, odds ratio with 95% CI, the significance value of association (P) and the estimated transmission rate (t) with its standard deviation (SD) are provided. The transmission rate (t) was estimated from all trios and duos using a maximum likelihood method (8).

The values in bold are statistically significant findings.

Variants in \textit{GDNF} and \textit{NRTN} have been identified in only a small minority of individuals with HSCR, and in almost all of those individuals, a variant was also identified in \textit{RET} or another HSCR gene, suggesting that mutation of one of the ligands is not sufficient by itself to cause disease [Angrist et al 1996, Hofstra et al 1996, Ivanchuk et al 1996, Salomon et al 1996, Doray et al 1998, Eketjall & Ibanez 2002].

**EDNRB and related genes.** Components of another cell signaling pathway that probably interacts with the RET pathway during enteric neural crest-colonization have been implicated in HSCR [Carrasquillo et al 2002]; these include the endothelin receptor type B (\textit{EDNRB}) and its ligand, endothelin-3 (\textit{EDN3}). Synthesis of the mature active form of endothelin-3 requires post-translational modification by endothelin-converting enzyme 1 (encoded by \textit{ECE1}).

\textit{EDNRB} and \textit{EDN3} pathogenic variants probably account for approximately 10% of individuals with HSCR [Amiel et al 1996, Kusafuka et al 1996, Svensson et al 1999]. Within the Mennonite community, however, a significant proportion of affected individuals have a missense pathogenic variant in \textit{EDNRB}, representing a founder variant, and some of these individuals have manifestations of Waardenburg syndrome type 4 (WS4) [Puffenberger et al 1994]. In general, individuals with a heterozygous pathogenic variant in \textit{EDNRB} or \textit{EDN3} present with HSCR or occasionally features of WS4, while those with homozygous pathogenic variants in either gene are more likely to have more severe manifestations of WS4 [Verheij et al 2002].
A pathogenic variant in *ECE1* has been reported in only one individual, who also had craniofacial anomalies and a heart defect [Hofstra et al 1999].

**NRG signaling pathway.** A genome-wide association study identified two polymorphisms in *NRG1* (rs16879552 T→C; rs7835688 G→C) as susceptibility loci for the development of HSCR in a large Chinese sample [Garcia-Barcelo et al 2009] (see table). The risk of developing HSCR goes up significantly when one or more of the *NRG1* variants are found in conjunction with the common *RET* benign variant, rs2435357 [Garcia-Barcelo et al 2009]. These variants did not appear to confer increased susceptibility to HSCR in a large European population, however [Kapoor et al 2015]. *NRG1* encodes a growth factor that is expressed in intestinal mucosa and enteric cells. Coding pathogenic variants with functional implications in *NRG1* have also been identified in individuals with HSCR, and in most cases have been associated with short segment, non-syndromic disease, although at least one individual had long segment disease and a few had other congenital anomalies [Tang et al 2012b]. Copy number variants (mostly deletions) in *NRG3* (a paralog of *NRG1*) have been implicated in HSCR pathogenesis [Borrego et al 2013], but most reported deletions have been small, intragenic deletions rather than large multi-gene deletions. One case was reported to have a recto-cutaneous fistula [Tang et al 2012a].

**SEMA3 gene complex.** Benign variants in the Semaphorin 3 cluster of genes on chromosome 7, especially rs11766001 A→C (see table), have been implicated as conferring increased susceptibility to development of HSCR, especially in conjunction with *RET* benign variants [Jiang et al 2015]. These proteins are involved in neuronal migration, proliferation, survival, and/or axonal guidance. The 4 class 3 Semaphorins are expressed in gut tissues during development, and SEMA3C and SEMA3D are both expressed in the enteric nervous system in several animal models [Jiang et al 2015]. Likely pathogenic variants have also been described in two genes within this cluster, *SEMA3C* and *SEMA3D*, and are enriched in those with HSCR over controls [Jiang et al 2015].

Based on genetic analyses, Kapoor and others [2015] have shown that the risk of developing HSCR increases with the number of susceptibility alleles that an individual harbors. With sophisticated sequencing methods now available, it is likely that additional susceptibility loci will be identified. Thus, a combination of common variants, such as those at the *RET*, *SEMA3*, and *NRG1* loci, together with rare deleterious variants, may be shown to underlie the complex genomic disorder, HSCR.

**References**


