Hirschsprung disease, associated syndromes, and genetics: a review

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Abstract

Hirschsprung disease (HSCR, aganglionic megacolon) is the main genetic cause of functional intestinal obstruction with an incidence of 1/5000 live births. This developmental disorder is a neurocristopathy and is characterised by the absence of the enteric ganglia along a variable length of the intestine. In the last decades, the development of surgical approaches has dramatically decreased mortality and morbidity, which has allowed the emergence of familial cases. HSCR appeared to be a multifactorial malformation with low, sex dependent penetrance and variable expression according to the length of the aganglionic segment, suggesting the involvement of one or more gene(s) with low penetrance. So far, eight genes have been found to be involved in HSCR. This frequent congenital malformation now stands as a model for genetic disorders with complex patterns of inheritance.

Keywords: Hirschsprung disease; aganglionic megacolon; genetics

Harald Hirschsprung first described in 1888 two unrelated boys who died from chronic severe constipation with abdominal distension resulting in congenital megacolon. The absence of intramural ganglion cells of the myenteric and submucosal plexuses (Auerbach’s and Meissner’s plexuses, respectively) downstream from the dilated part of the colon was considered to be the cause of the disease in the 1940s. This allowed simple and reliable diagnostic confirmation from rectal suction biopsies using histochemical staining for acetylcholinesterase (AchE). In 1948, Swenson and Bill developed a surgical procedure and the survival of patients uncovered familial transmission of HSCR. In 1973, Bolande proposed the term neurocristopathy for syndromes or tumours involving neural crest (NC) cells. HSCR resulting from an anomaly of the enteric nervous system (ENS) of NC origin is therefore regarded as a neurocristopathy.

HSCR occurs as an isolated trait in 70% of patients, is associated with a chromosomal abnormality in 12% of cases, and with additional congenital anomalies in 18% of cases. In the latter group of patients, some monogenic syndromes can be recognised. Isolated HSCR appears to be a multifactorial malformation with low, sex dependent penetrance, variable expression according to the length of the aganglionic segment, and suggesting the involvement of one or more gene(s) with low penetrance. These parameters must be taken into account for accurate evaluation of the recurrence risk in relatives.

Segregation analyses suggested an oligogenic mode of inheritance in isolated HSCR. With a relative risk as high as 200, HSCR is an excellent model for the approach to common multifactorial diseases. So far, genetic heterogeneity in HSCR has been shown with eight specific genes involved. The major susceptibility gene is RET, which is also involved in multiple endocrine neoplasia type 2 (MEN 2). The identification of modifier genes is currently under way. The aim of this paper is to review the clinical data on syndromic HSCR and the molecular findings over the last 10 years.

Definition and classification

HSCR is a congenital malformation of the hindgut characterised by the absence of parasympathetic intrinsic ganglion cells in the submucosal and myenteric plexuses. It is regarded as the consequence of the premature arrest of the craniocaudal migration of vagal neural crest cells in the hindgut between the fifth and twelfth week of gestation to form the enteric nervous system (ENS) and is therefore regarded as a neurocristopathy.

While the internal anal sphincter is the constant inferior limit, patients can be classified as short segment HSCR (S-HSCR, 80% of cases) when the aganglionic segment does not extend beyond the upper sigmoid, and long segment HSCR (L-HSCR, 20% of cases) when aganglionosis extends proximal to the sigmoid. Four HSCR variants have been reported: (1) total colonic aganglionosis (TCA, 3-8% of cases), (2) total intestinal HSCR (3) ultra short segment HSCR involving the distal rectum below the pelvic floor and the anus, and (4) suspended HSCR, a controversial condition, where a portion of the colon is aganglionic above a normal distal segment.
Clinical features and diagnosis
In most cases, the diagnosis of HSCR is made in the newborn period owing to intestinal obstruction with the following features: (1) failure to pass meconium within the first 48 hours of life, (2) abdominal distension that is relieved by rectal stimulation or enemas, (3) vomiting, and (4) neonatal enterocolitis. Some patients are diagnosed later in infancy or in adulthood with severe constipation, chronic abdominal distension, vomiting, and failure to thrive. Finally, although a rare presentation, unexplained perforation of the caecum or appendix should make the diagnosis be considered.

On abdominal x ray, a distended small bowel and proximal colon with an empty rectum are common findings. The classical image is a dilated proximal colon with the aganglionic cone narrowing towards the distal gut. On barium enema a small rectum with uncoordinated contractions is seen. The transition zone represents the site where the narrow aganglionic bowel joins the dilated ganglionic bowel. On a plain x ray taken later, delayed barium evacuation is observed. Anorectal manometry shows absence of relaxation of the internal sphincter in response to rectal distension. The reliability of this test becomes excellent from day 12 after birth when the normal rectoenteric reflex is present. Suction rectal biopsy confirms the diagnosis in most cases, but a full thickness rectal biopsy is needed for diagnosis of HSCR. Furthermore, extramucosal serial biopsies will be required at laparotomy to define the proximal limit of the aganglionic segment.

Differential diagnosis
Other causes of intestinal obstruction should be considered when abdominal distension and failure to pass meconium occur in a newborn infant, namely: (1) meconium ileus resulting from cystic fibrosis, (2) intestinal malformations such as lower ileal and colonic atresia, isolated or occasionally associated with HSCR, intestinal malrotation, or duplication, (3) ENS anomalies grouped together as chronic intestinal pseudo-obstruction syndromes, and (4) functional intestinal obstruction resulting from maternal infection, maternal intoxication, or congenital hypothyroidism.

Treatment and prognosis
The treatment of HSCR is surgical. After careful preoperative management, the principle is to place the normal bowel at the anus and to release the tonic contraction of the internal anal sphincter. Since the initial protocol of Swenson described in 1948, a series of operative approaches have been developed, such as the Soave and Duhamel procedures. A one-stage procedure is possible when diagnosis is made early, before colonic dilatation. Otherwise, a primary colostomy is required. Fistula or stenosis of the anastomosis and enterocolitis are the main short term complications. Long term complications include chronic constipation (10-15%) and soilings. Laparoscopic techniques have recently been proposed in HSCR surgery. Mortality is under 6% since the 1980s and may be related to short term complications or caused by the associated malformations. However, the treatment of children with TCA is still hazardous. Epidemiology
The incidence of HSCR is estimated at 1/5000 live births. However, the incidence varies significantly among ethnic groups (1.5, 2.1, and 2.8 per 10000 live births in Caucasians, African-Americans, and Asians, respectively). S-HSCR is far more frequent than L-HSCR (80% and 20%, respectively). There is a sex bias with a preponderance of affected males and a sex ratio of 4:1. Interestingly, the male:female ratio is significantly higher for S-HSCR than for L-HSCR (table 1). HSCR occurs as an isolated trait in 70% of cases. A chromosomal abnormality is associated with it in 12% of cases, trisomy 21 being by far the most frequent (>90%). Associated congenital anomalies are found in 18% of HSCR patients. The ones occurring at a frequency above that expected by chance include gastrointestinal malformation, cleft palate, polydactyly, cardiac septal defects, and craniofacial anomalies. The higher rate of associated anomalies in familial cases than in isolated cases (39% versus 21%) strongly suggests syndromes with Mendelian inheritance. Assessment of all HSCR patients should include a careful evaluation for recognisable syndromes by a trained dysmorphologist.

Chromosomal anomalies
A large number of chromosomal anomalies have been described in HSCR patients. Free trisomy 21 (Down syndrome) is by far the most frequent, involving 2-10% of ascertained HSCR cases. In these cases, both the unbalanced sex ratio (5.5:10.5 male:female) and the predominance of S-HSCR are even greater than in isolated HSCR. Overexpression of genes on chromosome 21 predisposing to HSCR has been hypothesised and a susceptibility gene mapping to 21q22 postulated in a Mennonite kindred. However, these data were not confirmed in other populations. Hitherto, mutations in genes predisposing to HSCR, namely RET, EDNRB, and GDNF, respectively, have been found in only three patients with Down syndrome and HSCR. Some chromosomal interstitial deletions reported in combination with HSCR have been important for the identification of HSCR predisposing genes, namely (1) 10q11.2 interstitial deletion observed in a few patients with...
L-HSCR or TCA, leading to the mapping and identification of the first gene for HSCR (RET); (2) 13q22.1-32.1 interstitial deletion in patients with S-HSCR encompassing a second gene (EDNRB); (3) 2q22-23 interstitial deletion in patients with S-HSCR (table 2), leading to the identification of the SIP1 gene (SMAD interacting protein 1).

Rarer chromosomal anomalies reported in combination with HSCR are summarised in table 2. DiGeorge syndrome, mosaic trisomy 8, XXY chromosomal constitution, partial duplication of chromosome 2q, tetrasomy 9p, and 20p deletion each have been observed once with HSCR.

### Syndromes and associated anomalies

Both the recognition of known entities and the delineation of novel ones including HSCR as a feature are of importance for disease prognosis, accurate genetic counselling, and search for candidate genes. Syndromes associated with HSCR can be classified as: (1) pleiotropic neurocristopathies, (2) syndromes with HSCR as a mandatory feature, (3) occasional association with recognisable syndromes, and (4) miscellaneous observations (table 3).

### Neurocristopathies

The NC is a transient and multipotent embryonic structure that gives rise to neuronal, endocrine and paraendocrine, craniofacial, and pigmentary tissues. Neurocristopathies encompass tumours, malformations, and single or multifocal abnormalities of tissues mentioned above in various combinations. MEN 2, conotruncal heart defects, congenital central hypotension, and

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<th>MIM</th>
<th>Key features</th>
<th>References</th>
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<td>WS4 (Shah-Waardenburg)</td>
<td>277580</td>
<td>Pigmentary anomalies (white forelock, iris hypoplasia, patchy hypopigmentation), deafness</td>
<td>53-57</td>
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<td>Yeminite deaf-blind-hypopigmentation</td>
<td>601706</td>
<td>Hearing loss, eye anomalies (microcornea, coloboma, nyctagmus), pigmentary anomalies</td>
<td>60</td>
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<td>RADS</td>
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<td>Hearing loss, hypopigmentation of the skin and retina</td>
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<td>Piebaldism</td>
<td>172800</td>
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<td>209880</td>
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<td>MEN2A</td>
<td>171400</td>
<td>Medullary thyroid carcinoma, phaeochromocytoma, hyperplasia of the parathyroid</td>
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<td>Riley-Day</td>
<td>223900</td>
<td>Autonomic nervous system anomalies</td>
<td></td>
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<td>HSCR mandatory</td>
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<tr>
<td>Goldberg-Shprintzen</td>
<td>235730</td>
<td>Cleft palate, hypotonia, microcephaly, mental retardation, dysmorphic facial features</td>
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<tr>
<td>HSCR with limbs anomalies</td>
<td>235740</td>
<td>Polydactyly, unilateral renal agenesis, hypertelorism, deafness</td>
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<td>235750</td>
<td>Postaxial polydactyly, ventricular septal defect</td>
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<td>235760</td>
<td>Hypoplasia of distal phalanges and nails, dysmorphic features</td>
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<td>602111</td>
<td>Preaxial polydactyly, heart defects, laryngeal anomalies</td>
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<td>306980</td>
<td>Brachydactyly type D</td>
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<td>BRESHEK</td>
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<tr>
<td>235740</td>
<td>Brain abnormalities, Retardation, Ectodermal dysplasia, Skeletal malformation, Hirschsprung disease, Ear/eye anomalies, Kidney dysplasia</td>
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<tr>
<td>Mesomelic dysplasia, Werner type</td>
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<tr>
<td>235730</td>
<td>Mesomelia, polydactyly</td>
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<tr>
<td>HSCR occasionally associated</td>
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<tr>
<td>Bardet-Biedl and/or</td>
<td>209900</td>
<td>Pigmentary retinopathy, obesity, hypogonitalism, mild mental retardation, postaxial polydactyly</td>
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<td>Kaufman-McKusick</td>
<td>236700</td>
<td>Hydrometrocolpos, postaxial polydactyly, congenital heart defect</td>
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<td>Smith-Lemli-Opitz</td>
<td>270400</td>
<td>Growth retardation, microcephaly, mental retardation, hypoplasias, 2–3 toes syndactyly, dysmorphic features</td>
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<tr>
<td>Cartilage-hair hypoplasia</td>
<td>250250</td>
<td>Short limb dwarfism, metaphyseal dysplasia, immunodeficiency</td>
<td>97</td>
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<tr>
<td>HSCR rarely associated</td>
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<tr>
<td>Fukuyama congenital muscular dystrophy</td>
<td>253800</td>
<td>Muscular dystrophy, polymicrogyria, hydrocephalus, MR, seizures</td>
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<tr>
<td>Clayton-Smith</td>
<td>258840</td>
<td>Dysmorphic features, hypoplastic toes and nails, ichthyosis</td>
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<tr>
<td>Kaplan</td>
<td>204100</td>
<td>Agenesis of corpus callosum, adducted thumbs, ptosis, muscle weakness</td>
<td>102</td>
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<tr>
<td>Okamoto</td>
<td>308840</td>
<td>Hydrocephalus, cleft palate, corpus callosum agenesis</td>
<td>103</td>
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<tr>
<td>Miscellaneous associations</td>
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<tr>
<td>Pallister-Hall (CAVE)</td>
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<td>Fryns</td>
<td>229850</td>
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<td>Aarskog</td>
<td>100050</td>
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<tr>
<td>Jeune asphyxiating thoracic dystrophy</td>
<td>208500</td>
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<tr>
<td>Frontonasal dysplasia</td>
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<td>Osteopetrosis</td>
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<td>Goldenhar</td>
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<tr>
<td>Lesch-Nyhan</td>
<td>308000</td>
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<tr>
<td>Rubinstein-Taybi</td>
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<tr>
<td>Toriello-Carey</td>
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<tr>
<td>SEMIDJL</td>
<td>271640</td>
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</table>
Waardenburg syndrome illustrate each of these categories and can be associated with HSCR.

*Multiple endocrine neoplasia type 2 (MEN 2).*

The MEN 2 syndromes include three types of cancer predisposition with an autosomal dominant mode of inheritance: familial medullary thyroid carcinoma (FMTC), MEN type 2A, (MEN 2A) and type 2B (MEN 2B). MEN 2A is defined by an age related predisposition to medullary thyroid carcinoma (MTC, 70% by the age of 70 years), pheochromocytoma (50% of cases), and hyperplasia of the parathyroid glands (15-35%). In addition to MTC and pheochromocytoma, subjects with MEN 2B present with oral neuromas, marfanoid habitus, and hyperganglionosis of the hindgut.45 Germline missense mutations of the RET gene have been identified in MEN 2A, MEN 2B,and FMTC. Both FMTC and MEN 2A can be associated with HSCR in some families.46–50 Interestingly, these families present a germline RET mutation of the MEN 2A or FMTC type (see below).44–50 This raises the question of whether all subjects with HSCR, regardless of a non-contributory family history, should be screened for RET exon 10 and 11 mutations to rule out cancer predisposition (3/160 cases in our series, C609W, C611R, and C620R RET gene mutations).

**Waardenburg syndromes (WS) and related pigmentary anomalies**

WS, an autosomal dominant condition, is by far the most frequent condition combining pigmentary anomalies and sensorineural deafness (1/50 000 live births and 2-5% of all congenital deafness), resulting from the absence of melanocytes of the skin and the stria vascularis of the cochlea.51 WS is clinically and genetically heterogeneous (MIM 193500, MIM 148820, MIM 193510).52 The combination of HSCR with WS defines the WS4 type (Shah-Waardenburg syndrome, MIM 277580), a genetically heterogeneous condition. Indeed, homozygous mutations of the endothelin pathway53–56 and heterozygous SOX10 mutations have been identified in WS4 patients (see below).57 Patients carrying a SOX10 mutation may also present with CNS involvement including seizures, ataxia, and demyelinating peripheral and central neuropathies.58–60

Pigment related syndromes that may include HSCR include: (1) Yemenite deaf-blind hypopigmentation syndrome (MIM 601706). A SOX10 mutation has been reported in one of these families;61 (2) Black locks-Albinism-Deafness Syndrome (BADS, MIM 227010) with TCA-HSCR reported in one case;62 (3) aganglionic megacolon associated with familial piebaldism (MIM 172800);63 (4) HSCR and profound congenital deafness but with no other WS features has also been reported.64

**Congenital central hypventilation syndrome (CCHS, MIM 209880)**

Initially termed Ondine’s curse, CCHS is a rare, life threatening condition characterised by abnormal ventilatory response to hypoxia and hypercapnia owing to failure of autonomic respiratory control.65 CCHS patients often present symptoms resulting from a broader dysfunction of the autonomic nervous system and neural crest derived tumours have also been observed.66–68 CCHS may be a polygenic disorder with a major locus being involved.66 Recurrence risk in sibs is estimated as 5% with few multicase families reported.66 Haddad syndrome (MIM 209880) is defined by the combination of CCHS with HSCR and represents 14-20% of CCHS patients.66–71 In these cases, L-HSCR (including TCA) is by far the most frequent, and the sex ratio is equal, contrary to what is observed in isolated HSCR.72 Mutations of the RET and the endothelin signalling pathways have been identified in rare CCHS patients: a RET mutation inherited from a healthy parent in two patients with Haddad syndrome,72–74 a GDNF mutation inherited from a healthy mother in a CCHS patient,72 and an EDN3 mutation in a CCHS patient.75

**Other neurocristopathies**

Familial dysautonomia syndrome (FDS, Riley-Day syndrome, MIM 223900) has been reported once in association with HSCR. Although it could have arisen by chance alone, it is interesting to note that the FDS gene (IKBKAP) maps to 9q31 where a susceptibility locus for HSCR has been identified (see below). Other occasional associations reported so far include cleft lip with or without cleft palate, neural crest derived tumours (neuroblastoma, ganglioneuroblastoma),75–76 neural tube defects (myelomeningocoele),77 and neurofibromatosis type 1.78 The significance of these associations is not yet established.

**SYNDromes WITH HSCR AS A MANDATORY FEATURE**

**Goldberg-Shprintzen syndrome (MIM 235730)**

This rare, probably autosomal recessive, multiple congenital anomalies-mental retardation syndrome combines HSCR, cleft palate, hypotonia, microcephaly and mental retardation with or without facial dysmorphic features ( hypertelorism, thin upper lip, sparse hair).79 The observation of both ventricular dilatation and irregular density of white matter on brain imaging may suggest a neuronal migration defect. Several reports with variable association of microcephaly, iris coloboma, cleft palate, and mental retardation may be variants of this syndrome.80–82 In our opinion, patients with a SIP1 gene mutation have a different condition.

**HSCR with limb anomalies**

A series of rare syndromes with HSCR and distal limb anomalies (polydactyly or hypoplasia) have been reported. These are: (1) HSCR with polydactyly, unilateral renal agenesis, hypertelorism, and congenital deafness (MIM 235740);82 (2) HSCR, postaxial polydactyly, and ventricular septal defects (MIM 235750);83 (3) HSCR, hypoplasia of the distal phalanges and nails, and mild dysmorphic features (MIM 23
35760); (4) HSCR with preaxial polydactyly, heart defect, and laryngeal anomalies (MIM 604211); (5) HSCR with brachydactyly type D (MIM 306980); (6) HSCR with brachydactyly, macrocephaly, and vertebral anomalies; (7) BRESHEK syndrome, and (8) mesomelic dysplasia, Werner type.

Occasional association in recognisable syndromes

The occasional association of HSCR with three syndromes or groups of syndromes of autosomal recessive mode of inheritance may be of interest for the identification of susceptibility genes in HSCR.

McKusick-Kaufman syndrome (MKKS, MIM 236700) and Bardet-Biedl syndrome (BBS, MIM 209900)

MKKS is a rare condition characterised by hydrometrocolpos, postaxial polydactyly, and congenital heart defect. HSCR is found in 10% of cases.\(^{89}\) The MKKS disease causing gene on 20p12, encoding a chaperonin protein, has recently been identified.\(^{90}\)

BBS is characterised by progressive pigmented retinopathy, obesity, hypogenitalism, renal involvement (including cysts, renal cortical loss, or reduced ability to concentrate urine), mild mental retardation, and postaxial polydactyly of the hands and feet. BBS is genetically heterogeneous with at least five loci involved, mapped to chromosome arms 2q, 3p, 11q, 15q, and 16q. HSCR has been reported in several BBS cases.\(^{91,92}\) Recently, mutations in the MKKS gene were identified in some BBS patients confirming clinical overlap at the molecular level.\(^{13}\)

Smith-Lemli-Opitz syndrome (SLO, MIM 270400)

SLO is characterised by pre- and postnatal growth retardation and microcephaly, severe mental retardation, facial dysmorphic features, hypospadias, and syndactyly between toes 2 and 3. SLO results from cholesterol metabolic impairment with mutation of the 7-dehydrocholesterol reductase gene (DHCR7, chromosome 11q12-q13).\(^{94,95}\) HSCR is observed in a significant number of severe SLO patients.\(^{96}\)

Cartilage-hair hypoplasia syndrome (CHH, MIM 250250)

This skeletal dysplasia, first described in the Old Order Amish community, combines metaphyseal dysplasia with short limb dwarfism, fine, sparse, and blond hair, transient macrocytic anaemia, and immunodeficiency. HSCR is associated with it in approximately 10% of the cases.\(^{97}\) The gene (RMRP) has been mapped to chromosome 9p13.\(^{98}\) Interestingly, HSCR has been reported in the Holmgren-Connor syndrome (MIM 211120), which may be allelic to CHH.

Molecular genetics

Segregation studies in non-syndromic HSCR have shown that the recurrence risk in sibs varies from 1% to 33% depending on the gender and the length of the aganglionic segment in the proband and the gender of the sib (table 1).\(^{7,14}\) Consequently, HSCR has been assumed to be a sex modified multifactorial disorder, the effect of genes playing a major role as compared to environmental factors (relative risk of 200).

So far, eight genes are known to be involved in HSCR in humans, namely the proto-oncogene RET (RET), glial cell line derived neurotrophic factor (GDNF), neurturin (NTN), endothelin B receptor (EDNRB), endothelin 3 (EDN3), endothelin converting enzyme 1 (ECE1), SOX10, and SIP1 genes (table 4).
### Table 4 Genes involved in HSCR in humans and known mouse models of megacolon

<table>
<thead>
<tr>
<th>Gene</th>
<th>Map location</th>
<th>Mode of inheritance</th>
<th>Phenotype in mutants</th>
<th>Frequency of mutation in heterozygous</th>
<th>Refs</th>
<th>Natural mutant</th>
<th>Knock-out</th>
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<tbody>
<tr>
<td>RET</td>
<td>10q11.2</td>
<td>AD</td>
<td>HSCR</td>
<td>50% familial cases</td>
<td>119</td>
<td>—</td>
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<td>108</td>
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<td>GDNF</td>
<td>5p13</td>
<td>AD</td>
<td>HSCR</td>
<td>15% sporadic cases</td>
<td>31, 142, 143</td>
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<td>132–134</td>
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<td>NTN</td>
<td>22q13</td>
<td>AD</td>
<td>WS4</td>
<td>5 cases</td>
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<td>Dom (AD)</td>
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<td>164</td>
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<td>ECE1</td>
<td>1p36</td>
<td>AD</td>
<td>HSCR</td>
<td>57, 59, 165</td>
<td>5, 157–160</td>
<td>i' (AR)</td>
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<td>EDNRB</td>
<td>13q22</td>
<td>AR/AD</td>
<td>WS4/HSCR</td>
<td>5%</td>
<td>154</td>
<td>S</td>
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<td>EDN3</td>
<td>20q13</td>
<td>AR/AD</td>
<td>HSCR</td>
<td>&lt;5%</td>
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<td>HSCR</td>
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<td>CF and cardiac defect</td>
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<tr>
<td>SIP1</td>
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<td>HSCR, MR, facial dysmorph</td>
<td>6 cases</td>
<td>119</td>
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</table>


### THE RET SIGNALLING PATHWAY

The first susceptibility locus was mapped to 10q11.2 in multigenerational families segregating HSCR as an incompletely penetrant autosomal dominant trait.112 113 This region had been targeted because of the observation of an interstitial deletion of chromosome 10q11.2 in patients with TCA and mental retardation.15 The proto-oncogene RET, identified as disease causing in MEN 2,114 115 and mapping in 10q11.2, was regarded as a good candidate gene owing to the concurrence of MEN 2A and HSCR in some families and the expression in neural crest derived cells. Consequently, RET gene mutations were identified in HSCR patients.116–117 RET is a 1114 amino acid transmembrane receptor with a cadherin-like extracellular domain, a cysteine rich region, and an intracellular tyrosine kinase domain.118 Expression and penetrance of a RET mutation is variable and sex dependent within HSCR families. In large series, the estimated penetrance is 72% in males and 51% in females.119 Over 80 mutations have been identified including large deletions encompassing the RET gene, microdeletions and insertions, nonsense, missense and splicing mutations.116–122 There is no mutational hot spot at variance with MEN 2A, where mutations occur in a cluster of six cysteines (exon 10, residues 609, 611, 618, 620; exon 11, residues 630,634),114 115 and MEN 2B where the mutation is almost unique (M918T, exon 16, tyrosine kinase domain).122–124 In vitro, MEN 2 mutations have been shown to be activating mutations leading to constitutive dimerisation of the receptor and to transformation,125 while haploinsufficiency is the most likely mechanism for HSCR mutations.126–128 Biochemical studies showed variable consequences of some HSCR mutations (misfolding, failure to transport the protein to the cell surface, abolished biological activity).127 128 However, a simple activating versus inactivating model of gene action is not sufficient to explain the concurrence of HSCR and MEN 2A in patients with a MEN 2A RET gene mutation.

Despite extensive mutation screening, a RET mutation is identified in only 50% of familial and 15–20% of sporadic HSCR cases.119 However, most families with few exceptions are compatible with linkage at the RET locus.129 Recent studies of known polymorphisms within the RET gene in a series of sporadic HSCR patients showed a significantly different distribution as compared to controls. Several polymorphic haplotypes could be associated with predisposition to HSCR.130 131 Epigenetic factors could also be involved.

GDNF, known as a major survival factor for many types of neurones, was shown to be the RET ligand by both phenotypic similarities between Ret−/− and Gdnf−/− knockout mice132–134 and Xenopus embryo bioassays.135 GDNF is a TGF-B related 211 residue protein, proteolytically cleaved to a 134 residue mature protein that homodimerises. To activate RET, GDNF needs the presence of a novel glycosylphosphatidylinositol (GPI) linked coreceptor GFRA1.136 137 Four related GPI linked coreceptors, GFRA1–4,138 and four related soluble growth factor ligands of RET have been identified, namely GDNF, NTN,139–141 persephin (PSPN),142 and artemin.143 Specific combinations of these proteins are necessary for development and maintenance of both central and peripheral neurones and all can signal through RET. GDNF mutations have been identified in only six HSCR patients to date, and can be regarded as a rare cause of HSCR (<5%).144–146 Moreover, GDNF mutations may not be sufficient to lead to HSCR since four out of six patients have additional contributory factors, such as RET mutations or trisomy 21.145 146 Similarly, a NTN mutation has been identified in one family, in conjunction with a RET mutation.144 Finally, although Gfra1 homozygous knockout mice are phenotypically very similar to Ret and Gdnf−/− mice, no GFRA1 mutations have been identified in HSCR patients.145 146

### THE ENDOTHELIN SIGNALLING PATHWAY

The endothelin pathway was first studied for its vasoconstrictive effect and putative role in hypertension. EDNRB and EDNRA are G protein coupled heptahelical proteins that transduce signals through the endothelins (EDN1, 2, 3).149 150
A susceptibility locus for HSCR in 13q22 was suggested for three main reasons: (1) a significant lod score at 13q22 in a large inbred Old Order Mennonite community with multiple cases of HSCR, (2) de novo interstitial deletion of 13q22 in several patients with HSCR, and (3) synteny between the murine locus for piebald-lethal ($\gamma$), a model of aganglionosis, and 13q22 in humans. The critical role of the endothelin pathway in HSCR was shown by the finding that piebald-lethal was allelic to the Ednrb knockout mouse and harboured an Ednrb mutation (table 4). Subsequently, an EDNRB missense mutation was identified in the Mennonite kindred (W276C). However, the W276C mutation was neither necessary (affected wild type homozygotes) nor sufficient (unaffected mutant homozygotes) to cause HSCR, and penetrance was sex dependent (greater in males than in females). piebald-lethal was considered a mouse model for WS4 in humans and some of the affected Mennonite subjects had pigmentary anomalies and sensorineural deafness in addition to HSCR. This prompted a screen of the EDNRB gene in WS4 and homozygous mutations in a fraction of WS4 families were found. At the same time, an Ednrb mutation was identified in the lethal spotting (5) normal mouse model for WS4 and EDN3 homozygous mutations were identified in WS4 in humans (table 4).

Both EDNRB and EDN3 were screened in large series of isolated HSCR patients. While EDN3 mutations were seldom found, EDNRB mutations were identified in approximately 5% of the patients. It is worth mentioning that the penetrance of EDN3 and EDNRB heterozygous mutations is incomplete in those HSCR patients, de novo mutations have not hitherto been observed, and that S-HSCR is largely predominant. Interstitial 13q22 deletions encompassing the EDNRB gene in HSCR patients make haploinsufficiency the most likely mechanism for HSCR (table 2). Although EDNRB binds all three endothelins, the similarity of phenotype of the Ednrb knockout mice to that of the Edn3 knockout mice suggests that EDNRB's major ligand is EDN3 in neural crest derived cells.

Preproendothelins are proteolytically cleaved by two related membrane bound metalloproteases to give rise to the mature 21 residue endothelin. Ece1 processes only Edn1 and Edn3. Ece1 knockout mice show craniofacial defects and cardiac abnormalities in addition to colonic aganglionosis. A heterozygous ECE1 mutation has been identified in a patient combining HSCR and craniofacial and cardiac defects (R742C).

SOX10
The last de novo mouse model for WS4 in human is dominant megalon (Dom), homzygous Dom mutation being embryonic lethal. The Dom gene is Sox10, a member of the SRY (sex determining factor)-like, high mobility group (HMG) DNA binding proteins. Subsequently, heterozygous SOX10 mutations have been identified in familial and isolated patients with WS4 (including de novo mutation). At least some mutations disrupt the DNA binding domain and may lead to a loss of function allele, so that again haploinsufficiency is the most likely mechanism for HSCR. Others disrupt the transactivation domain and may result in a dominant negative effect. These latest mutations were identified in patients presenting neurological impairment in addition to HSCR and pigmentary anomalies. Penetrance appears to be high, although sibs sharing a mutation and discordant for HSCR have been described in one family. Therefore, SOX10 is unlikely to be a major gene in isolated HSCR.

**Interaction between pathways**
Ret and Ednrb signalling pathways were considered biochemically independent. However, G protein coupled receptors and receptor tyrosine kinases could be engaged in crosstalk. Moreover, an HSCR patient heterozygous for weak hypomorphic mutations in both RET and EDNRB has recently been reported. Each mutation was inherited from a healthy parent. Sox10 is involved in cell lineage determination and is capable of transactivating MITF synergistically with PAX3. Similarly, Ednrb transcripts are either absent or drastically reduced in Dom +/− and +/+ mice, respectively. Therefore, the reduced expression of Ednrb in the dom mouse could arise either from a direct effect of Sox10 or from an indirect effect on a subset of NC cells of common faith.

Taken all together, several general comments can be made. RET is the major gene in HSCR with a heterozygous mutation found in 50% of familial cases and 15–20% of isolated cases. RET mutation penetrance is incomplete and sex dependent. Genotype-phenotype correlation is poor. HSCR is genetically heterogeneous and results from mutations in distinct pathways. Some patients with mutations in more than one HSCR susceptibility gene are known (RET + GDNF, RET + NTF, RET + EDNRB).

**Multigenic inheritance of Hirschsprung disease**
As mentioned above, RET plays a key role in non-syndromic HSCR genesis and multiple genes may be required to modulate clinical expression. On the other hand, genetic heterogeneity, where mutation in one of several genes is sufficient for phenotypic expression of HSCR, has been reported (RET, EDNRB, EDN3, ECE1, SIP1). However, the observation of non-random association between HSCR, RET, and chromosome 21q22 in the Mennonite population where HSCR imperfectly segregates with an EDNRB mutation favours multigenic inheritance resulting from the cumulative effects of multiple mutations. According to the segregation analysis where an autosomal dominant model in L-HSCR and a multifactorial model in S-HSCR were more likely, two approaches have been chosen to test these hypotheses in L-HSCR and S-HSCR independently.
LINKAGE ANALYSIS IN 12 HSCR FAMILIES WITH THREE OR MORE AFFECTED SUBJECTS IN TWO OR MORE GENERATIONS

L-HSCR is largely predominant in these families. All but one family showed linkage to the RET locus. Mutational analysis identified a nonsense or missense mutation at a highly conserved residue in six families, a splice mutation in two families, and no coding sequence variation in three families. Linkage to a novel locus in 9q31 was identified only in families with no or hypomorphic RET gene mutation. Therefore, a severe RET mutation may lead to phenotypic expression by haplinsufficiency, while hypomorphic RET mutations would require the action of other mutations.

A SIB PAIR ANALYSIS IN 49 FAMILIES WITH S-HSCR PROBANDS

This study shows that only three loci on chromosomes 3p21, 10q11, and 19q12 are both necessary and sufficient to explain the incidence and sib recurrence risk in HSCR. A multiplicative risk across loci with most affected subjects being heterozygotes at all three loci seems the best genetic model. Interestingly, marker analysis showed a significant parent of origin effect at the RET locus; 78% of shared RET alleles being maternally derived, which could explain the sex difference in HSCR expression. Finally, linkage to 9q31 was confirmed in the sib pairs with no or hypomorphic RET mutation.

Genetic counselling

HSCR is a sex modified, multifactorial, congenital malformation with an overall recurrence risk in sibs of the proband of 4% (relative risk=200). In isolated HSCR, adequate relative risk figures will be provided by taking into account the sex and length of the aganglionic segment in the proband and the gender of the sib (1-3%). According to the Carter paradox, the highest recurrence risk is for a male sib of a female proband with L-HSCR (table 1). Because of poor genotype-phenotype correlation so far, the benefit of mutation screening for HSCR patients appears low except for systematic testing of exon 10 and 11 of the RET gene owing to cancer predisposition of MEN 2A mutations. This, unfortunately, is not yet routine practice.

Many HSCR cases are associated with other congenital anomalies. In these cases, the long term prognosis is highly dependent on the severity of the associated anomalies. Several known syndromes have straight Mendelian inheritance. This emphasizes the importance of careful assessment by a clinician trained in syndromology of all newborns diagnosed with HSCR.

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Hirschsprung disease, associated syndromes, and genetics


Hirschsprung disease, associated syndromes, and genetics: a review

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