Review article

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.

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Harald Hirschsprung first described in 1888 two unrelated boys who died from chronic severe constipation with abdominal distension resulting in congenital megacolon.¹ 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.² This allowed simple and reliable diagnostic confirmation from rectal suction biopsies using histochemical staining for acetylcholinesterase (AChE).³ In 1948, Swenson and Bill developed a surgical procedure⁴ and the survival of patients uncovered familial transmission of HSCR.⁵ In 1973, Bolande⁶ 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.^{6,7}

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.^{8–13} 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.^{5,14} 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.¹⁴ 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.² 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.^{6,15} 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),¹⁶ (2) total intestinal HSCR when the whole bowel is involved,¹⁶ (3) ultra short segment HSCR involving the distal rectum below the pelvic floor and the anus,¹⁷ and (4) suspended HSCR, a controversial condition, where a portion of the colon is aganglionic above a normal distal segment.

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Table 1 Epidemiology and recurrence risk figures in HSCR¹⁴

	L-HSCR	S-HSCR
% probands	19	81
Sex ratio (male:female)	1.75	5.5
Genetic model	Dominant	Multigenic or recessive
Penetrance (%) (male:female)	52:40	17:4
Recurrence risk to sibs* (%)		
Male proband	17/13	5/1
Female proband	33/9	5/3

*Recurrence risk are given for male/female sibs respectively.

Clinical features and diagnosis

In most cases, the diagnosis of HSCR is made in the newborn period¹³ 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.¹⁸ 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.¹⁹ The reliability of this test becomes excellent from day 12 after birth when the normal rectoenteric reflex is present.²⁰ Suction rectal biopsy confirms the diagnosis in most cases,²¹ 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 1948,⁴ a series of operative approaches have been developed, such as the Soave and Duhamel procedures.²²⁻²³ 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.²⁴ Long term complications include chronic constipation (10-15%) and soiling.²⁵⁻²⁶ Laparoscopic techniques have recently been proposed in HSCR surgery.²⁷ Mortality is under 6% since the 1980s and may be related to short term complications or caused by the associated malformations.²⁵ However, the treatment of children with TCA is still hazardous.²⁸⁻²⁹

Epidemiology

The incidence of HSCR is estimated at 1/5000 live births.⁵ However, the incidence varies significantly among ethnic groups (1.5, 2.1, and 2.8 per 10 000 live births in Caucasians, African-Americans, and Asians, respectively).¹³ S-HSCR is far more frequent than L-HSCR (80% and 20%, respectively).⁸⁻¹⁰ There is a sex bias with a preponderance of affected males and a sex ratio of 4/1.¹⁴ Interestingly, the male:female ratio is significantly higher for S-HSCR than for L-HSCR (table 1).¹³⁻¹⁴

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.¹¹⁻¹² The higher rate of associated anomalies in familial cases than in isolated cases (39% versus 21%) strongly suggests syndromes with Mendelian inheritance.¹² 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.⁵⁻⁹⁻¹³ 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.³⁰ 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.³¹⁻³²

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

Table 2 Recurrent chromosomal anomalies with HSCR as a feature

Chromosome	Key features	Number of reports	Gene	References
Tri 21	Down syndrome, S-HSCR, 5.5 to 10.5 male:female sex ratio	2 to 10% of HSCR cases	?	5, 9–13
Del 10q11	Mental retardation, L-HSCR	2 cases	RET	33, 34
Del 13q22	Mental retardation, growth retardation, dysmorphic features, S-HSCR	7 cases	EDNRB	35–37
Del 2q22-q23	Postnatal growth retardation and microcephaly, mental retardation, epilepsy, dysmorphic features, HSCR*	3 cases	SIP1	38–41
Del 17q21		4 cases	?	
Dup 17q21-q23	MCA/MR	4 cases	?	
Tri 22pter-q11	Cat eye syndrome		?	

*Both S-HSCR and L-HSCR have been observed. Several patients presenting the same pattern of congenital malformations and normal chromosomes have been reported.³⁹

L-HSCR or TCA,^{33–34} 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)^{35–37}; (3) 2q22–23 interstitial deletion syndrome in patients with HSCR (table 2),^{38–40} leading to the identification of the SIP1 gene (SMAD interacting protein 1).⁴¹

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, conotruncal heart, and pigmentary tissues.⁷ Neurocristopathies encompass tumours, malformations, and single or multifocal abnormalities of tissues mentioned above in various combinations. MEN 2, conotruncal heart defects, congenital central hypoventilation, and

Table 3 Syndromes associated with HSCR (reprinted and adapted from Scriver CM et al, eds. "The metabolic and molecular bases of inherited diseases" 8th ed. Chap 251. New York: McGraw-Hill: 6231–55.)

Syndromes	MIM	Key features	References
<i>Neurocristopathy syndromes</i>			
WS4 (Shah-Waardenburg)	277580	Pigmentary anomalies (white forelock, iris hypoplasia, patchy hypopigmentation), deafness	53–57
Yemenite deaf-blind-hypopigmentation	601706	Hearing loss, eye anomalies (microcornea, coloboma, nystagmus), pigmentary anomalies	60
BADS	227010	Hearing loss, hypopigmentation of the skin and retina	61
Piebaldism	172800	Patchy hypopigmentation of the skin	62, 63
Haddad	209880	Congenital central hypoventilation	70, 71
MEN2A	171400	Medullary thyroid carcinoma, pheochromocytoma, hyperplasia of the parathyroid	43–50
Riley-Day	223900	Autonomic nervous system anomalies	
<i>HSCR mandatory</i>			
Goldberg-Shprintzen	235730	Cleft palate, hypotonia, microcephaly, mental retardation, dysmorphic facial features	79
	235740	Polydactyly, unilateral renal agenesis, hypertelorism, deafness	82
HSCR with limbs anomalies	235750	Postaxial polydactyly, ventricular septal defect	83
	235760	Hypoplasia of distal phalanges and nails, dysmorphic features	84
	604211	Preaxial polydactyly, heart defect, laryngeal anomalies	85
	306980	Brachydactyly type D	86
BRESHEK		Brain abnormalities, Retardation, Ectodermal dysplasia, Skeletal malformation, Hirschsprung disease, Ear/eye anomalies, Kidney dysplasia	87
Mesomelic dysplasia, Werner type		Mesomelia, polydactyly	170
<i>HSCR occasionally associated</i>			
Bardet-Biedl and/or	209900	Pigmentary retinopathy, obesity, hypogenitalism, mild mental retardation, postaxial polydactyly	91, 92
Kauffman-McKusick	236700	Hydrometrocolpos, postaxial polydactyly, congenital heart defect	89
Smith-Lemli-Opitz	270400	Growth retardation, microcephaly, mental retardation, hypospadias, 2–3 toes syndactyly, dysmorphic features	96
Cartilage-hair hypoplasia	250250	Short limb dwarfism, metaphyseal dysplasia, immunodeficiency	97
<i>HSCR rarely associated</i>			
Fukuyama congenital muscular dystrophy	253800	Muscular dystrophy, polymicrogyria, hydrocephalus, MR, seizures	99, 100
Clayton-Smith	258840	Dysmorphic features, hypoplastic toes and nails, ichthyosis	101
Kaplan	304100	Agenesis of corpus callosum, adducted thumbs, ptosis, muscle weakness	102
Okamoto	308840	Hydrocephalus, cleft palate, corpus callosum agenesis	103
<i>Miscellaneous associations</i>			
Pallister-Hall (CAVE)	140510		
Fryns	229850		
Aarskog	100050		
Jeune asphyxiating thoracic dystrophy	208500		
Frontonasal dysplasia	136760		
Osteopetrosis			
Goldenhar	164210		
Lesch-Nyhan	308000		
Rubinstein-Taybi	180849		
Toriello-Carey	217980		
SEMDJL	271640		

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.⁴² 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.⁴³⁻⁵⁰ 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 59}

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 families⁶⁰; (2) Black locks-Albinism-Deafness Syndrome (BADs, MIM 227010) with TCA-HSCR reported in one case⁶¹; (3) aganglionic megacolon associated with familial piebaldism (MIM 172800)^{62 63}; (4) HSCR and profound congenital deafness but with no other WS features has also been reported.⁶⁴

Congenital central hypoventilation 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 cell derived tumours have also been observed.⁶⁵⁻⁶⁸ CCHS may be a polygenic disorder with a major locus being involved.⁶⁹ Recurrence risk in sibs is estimated as 5% with few multicase families reported.⁶⁵ Haddad syndrome (MIM 209880) is defined by the combination of CCHS with HSCR and represents 14-20% of CCHS patients.^{70 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.⁷¹ 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 73} a *GDNF* mutation inherited from a healthy mother in a CCHS patient,⁷² and an *EDN3* mutation in a CCHS patient.⁷⁴

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 (myelomeningocele),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, prominent nose, synophrys, 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 81} 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

235760)⁸⁴; (4) HSCR with preaxial polydactyly, heart defect, and laryngeal anomalies (MIM 604211)⁸⁵; (5) HSCR with brachydactyly type D (MIM 306980)⁸⁶; (6) HSCR with brachydactyly, macrocephaly, and vertebral anomalies⁸⁷; (7) BRESHEK syndrome,⁸⁸ and (8) mesomelic dysplasia, Werner type.¹⁷⁰

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-Kauffman 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 pigmentary 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.⁹³

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.

MISCELLANEOUS OBSERVATIONS

For rare disorders, whether an association with HSCR observed once is meaningful or occurred by chance alone is not possible to decide. These conditions are summarised in

table 3 and can be classified as follows: (1) syndromes with muscular dystrophy,^{99–100} (2) syndromes with dermatological findings,¹⁰¹ and (3) syndromes with central nervous system anomalies.^{102–103} Other rare associations include the finding of HSCR with Fryns syndrome, Aarskog syndrome, Jeune asphyxiating thoracic dystrophy, frontonasal dysplasia, osteopetrosis, Goldenhar syndrome, Lesch-Nyhan syndrome, Rubinstein-Taybi syndrome, Toriello-Carey syndrome, Pallister-Hall syndrome, spondyloepimetaphyseal dysplasia with joint laxity (SEMDJL, MIM 271640), persistent müllerian duct syndromes, and asplenia with cardiovascular anomaly.

Isolated anomalies

A wide spectrum of additional isolated anomalies have been described among HSCR cases with an incidence varying from 5% to 30% according to series.^{8–9–11–104–107} No constant pattern is observed and these anomalies include distal limb, sensorineural, skin, central nervous system, genital, kidney, and cardiac malformations. However, cardiac defects, mostly atrio- or ventriculoseptal defects, are found with an incidence of 5% of cases of HSCR, excluding patients with trisomy 21. Renal dysplasia or agenesis was found in 4.4% in a series of 160 HSCR cases and may still be underestimated (personal data). This is of interest since homozygous knockout mice for genes involved in the *Ret* signalling pathway present with renal agenesis/dysplasia in addition to megacolon (see below).¹⁰⁸ Genital anomalies including hypospadias are reported in up to 2–3% of HSCR patients. Gastrointestinal malformations such as Meckel diverticulum, pyloric stenosis, single umbilical artery, inguinal hernia, or small bowel atresia are also found.^{109–111} Finally, facial dysmorphic features seem to be extremely frequent when looked for. These data highlight the importance of a careful assessment by a clinician trained in dysmorphology for all newborns diagnosed with HSCR. Skeletal x ray and cardiac and urogenital echographic survey should be systematically performed. The observation of one additional anomaly to HSCR should prompt chromosomal studies.

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).^{5–14} 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

Gene	Human					Mouse		
	Map location	Mode of inheritance	Phenotype in mutants	Frequency of mutation in heterozygotes	Refs	Natural mutant	Knock-out	Refs
<i>RET</i>	10q11.2	AD	HSCR	50% familial cases 15% sporadic cases	119	—	L Renal agenesis	108
<i>GDNF</i>	5p13	AD	HSCR	5 cases	31, 142, 143	—	L Renal agenesis	132–134
<i>NTN</i>	19p13	AD	HSCR	1 case	144	—	—	164
<i>SOX10</i>	22q13	AD	WS4	—	57, 59, 165	<i>Dom</i> (AD)	L Coat spotting	154
<i>EDNRB</i>	13q22	AR/AD	WS4/HSCR	5%	53, 157–160	<i>s^l</i> (AR)	S Coat spotting	155
<i>EDN3</i>	20q13	AR/AD	WS4/HSCR	<5%	156	<i>ls</i> (AR)	S Coat spotting	161
<i>ECE1</i>	1p36	AD	HSCR CF and cardiac defect	1 case	162	—	S Coat spotting CF defects	—
<i>SIP1</i>	2q22	Spo	HSCR, MR, facial dysmorphism	6 cases	38, 39, 41	—	—	—

AD: autosomal dominant, AR: autosomal recessive, Spo: sporadic, *s^l*: *Piebald lethal*, *ls*: *lethal spotting*, S: short segment megacolon, L: long segment megacolon, CF: craniofacial, MR: mental retardation.

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.³³ 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.^{119–121} 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),^{44–114–115} 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 mice^{132–134} and *Xenopus* embryo bioassays.¹³⁵ GDNF is a TGF- β 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 GFR α 1.^{136–137} Four related GPI linked coreceptors, *GFR α 1–4*,¹³⁸ and four related soluble growth factors ligands of *RET* have been identified, namely *GDNF*, *NTN*,¹³⁹ *persephin* (*PSPN*),¹⁴⁰ and *artemin*.¹⁴¹ 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%).^{31–142–143} 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.^{31–142} 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 *GFR α 1* mutations have been identified in HSCR patients.^{145–148}

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,^{30 151 152} (2) de novo interstitial deletion of 13q22 in several patients with HSCR,^{36 37 153} and (3) synteny between the murine locus for *piebald-lethal* (*s^l*), 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).¹⁵⁴ Subsequently, an *EDNRB* missense mutation was identified in the Mennonite kindred (W276C).⁵³ 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).⁵³ *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.^{30 151} This prompted a screen of the *EDNRB* gene in WS4 and homozygous mutations in a fraction of WS4 families were found.⁵⁴ At the same time, an *Edn3* mutation was identified in the *lethal spotting* (*ls*) natural mouse model for WS4¹⁵⁵ and *EDN3* homozygous mutations were identified in WS4 in humans (table 4).^{55 56}

Both *EDNRB* and *EDN3* were screened in large series of isolated HSCR patients. While *EDN3* mutations were seldom found,¹⁵⁶ *EDNRB* mutations were identified in approximately 5% of the patients.^{157–160} 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.¹⁶¹ A heterozygous *ECE1* mutation has been identified in a patient combining HSCR and craniofacial and cardiac defects (R742C).¹⁶²

SOX10

The last de novo mouse model for WS4 in human is *dominant megalon* (*Dom*), homozygous *Dom* mutation being embryonic lethal.¹⁶³ The *Dom* gene is *Sox10*, a member of the SRY (sex determining factor)-like, high mobility group (HMG) DNA binding proteins.¹⁶⁴ Subsequently, heterozygous *SOX10* mutations have been identified in familial and

isolated patients with WS4 (including de novo mutation).^{57 59 165} 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.⁵⁹ Penetrance appears to be high, although sibs sharing a mutation and discordant for HSCR have been described in one family.¹⁶⁵ 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.¹⁶⁶ Each mutation was inherited from a healthy parent.

Sox10 is involved in cell lineage determination and is capable of transactivating *MITF* synergistically with *PAX3*.¹⁶⁷ Similarly, *Ednrb* transcripts are either absent or drastically reduced in *Dom* ^{-/-} and *+/-* mice, respectively.¹⁶⁸ 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* + *NTN*, *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 GENERATIONS¹²⁹

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 haploinsufficiency, while hypomorphic *RET* mutations would require the action of other mutations.

A SIB PAIR ANALYSIS IN 49 FAMILIES WITH S-HSCR PROBANDS¹⁶⁹

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 (1-33%). 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 emphasises the importance of careful assessment by a clinician trained in syndromology of all newborns diagnosed with HSCR.

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