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 procedure[4] and the survival of patients uncovered familial transmission of HSCR.[5] In 1973, Bolande[6] 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.

Table 1 Epidemiology and recurrence risk figures in HSCR[14]

	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 period[13] 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
Neurocristopathy syndromes			
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, phaeochromocytoma, hyperplasia of the parathyroid	43–50
Riley-Day	223900	Autonomic nervous system anomalies	
HSCR mandatory			
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
HSCR occasionally associated			
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
HSCR rarely associated			
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
Miscellaneous associations			
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), phaeochromocytoma (50% of cases), and hyperplasia of the parathyroid glands (15-35%). In addition to MTC and phaeochromocytoma, 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 pathway[53–56] 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 families[60]; (2) Black locks-Albinism-Deafness Syndrome (BADS, MIM 227010) with TCA-HSCR reported in one case[61]; (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 anomalies[87]; (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 mullerian 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

	Human					Mouse		
Gene	Map location	Mode of inheritance	Phenotype in mutants	Frequence of mutation in heterozygotes	Refs	Natural mutant	Knock-out	Refs
RET	10q11.2	AD	HSCR	50% familial cases 15% sporadic cases	119	—	L Renal agenesis	108
GDNF	5p13	AD	HSCR	5 cases	31, 142, 143	—	L Renal agenesis	132–134
NTN	19p13	AD	HSCR	1 case	144	—	—	
SOX10	22q13	AD	WS4		57, 59, 165	Dom (AD)	L Coat spotting	164
EDNRB	13q22	AR/AD	WS4/HSCR	5%	53, 157–160	s^l (AR)	S Coat spotting	154
EDN3	20q13	AR/AD	WS4/HSCR	<5%	156	ls (AR)	S Coat spotting	155
ECE1	1p36	AD	HSCR CF and cardiac defect	1 case	162	—	S Coat spotting CF defects	161
SIP1	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-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 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 *GFRA1* 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 WS4[155] 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 GENERATIONS[129]

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 PROBANDS[169]

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.

1 Hirschsprung H. Stuhlträgheit neugeborener infolge von dilatation und hypertrophic des colons. *Jb Kinderheilkd* 1888;**27**:1.
2 Whitehouse F, Kernohan J. Myenteric plexuses in congenital megacolon; study of 11 cases. *Arch Intern Med* 1948;**82**: 75.
3 Meier-Ruge W, Lutterbeck PM, Herzog B, Morger R, Moser R, Scharli A. Acetylcholinesterase activity in suction biopsies of the rectum in the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 1972;**7**:11-17.
4 Swenson O. Early history of the therapy of Hirschsprung's disease: facts and personal observations over 50 years. *J Pediatr Surg* 1996;**31**:1003-8.
5 Bodian M, Carter C. A family study of Hirschsprung disease. *Ann Hum Genet* 1963;**26**:261.
6 Bolande RP. The neurocristopathies; a unifying concept of disease arising in neural crest maldevelopment. *Hum Pathol* 1973;**5**:409-29.
7 Le Douarin N, Kalcheim C. *The neural crest*. Cambridge: Cambridge University Press, 1999.
8 Passarge E. The genetics of Hirschsprung's disease. Evidence for heterogeneous etiology and a study of sixty-three families. *N Engl J Med* 1967;**276**:138-43.
9 Goldberg EL. An epidemiological study of Hirschsprung's disease. *Int J Epidemiol* 1984;**13**:479-85.
10 Garver KL, Law JC, Garver B. Hirschsprung disease: a genetic study. *Clin Genet* 1985;**28**:503-8.
11 Spouge D, Baird PA. Hirschsprung disease in a large birth cohort. *Teratology* 1985;**32**:171-7.
12 Brooks AS, Breuning MH, Meijers C. Spectrum of phenotypes associated with Hirschsprung disease: an evaluation of 239 patients from a single institution. *The Third International Meeting: Hirschsprung disease and related neurocristopathies, Evian, France*, 1998.
13 Torfs CP. An epidemiological study of Hirschsprung disease in a multiracial California population. *The Third International Meeting: Hirschsprung disease and related neurocristopathies, Evian, France*, 1998.
14 Badner JA, Sieber WK, Garver KL, Chakravarti A. A genetic study of Hirschsprung disease. *Am J Hum Genet* 1990;**46**:568-80.
15 Taraviras S, Pachnis V. Development of the mammalian enteric nervous system. *Curr Opin Genet Dev* 1999;**9**:321.
16 Nihoul-Fékété C, Ricour C, Martelli H, Lortat Jacob S, Pellerin D. Total colonic aganglionosis (with or without ileal involvement): a review of 27 cases. *J Pediatr Surg* 1986;**21**: 251.
17 Neilson IR, Yazbeck S. Ultrashort Hirschsprung's disease: myth or reality. *J Pediatr Surg* 1990;**25**:1135-8.
18 Parc R, Berrod JL, Tussiot J, Loygue J. Megacolon in adults. Apropos of 76 cases. *Ann Gastroenterol Hepatol (Paris)* 1984;**20**:133-41.
19 Emir H, Akman M, Sarimurat N, Kilic N, Erdogan E, Soylet Y. Anorectal manometry during the neonatal period: its specificity in the diagnosis of Hirschsprung's disease. *Eur J Pediatr Surg* 1999;**9**:101-3.
20 Lopez-Alonso M, Ribas J, Hernandez A, Anguita FA, Gomez de Terreros I, Martinez-Caro A. Efficiency of the anorectal manometry for the diagnosis of Hirschsprung's disease in the newborn period. *Eur J Pediatr Surg* 1995;**5**:160-3.
21 Kurer MH, Lawson JO, Pambakian H. Suction biopsy in Hirschsprung's disease. *Arch Dis Child* 1986;**61**:83-4.
22 Soave F. Hirschsprung's disease. Technique and results of Soave's operation. *Br J Surg* 1966;**53**:1023-7.
23 Newbern WR. Hirschsprung's disease - the Duhamel modification. *Am J Gastroenterol* 1967;**47**:61-8.
24 Hackam DJ, Filler RM, Pearl RH. Enterocolitis after the surgical treatment of Hirschsprung's disease: risk factors and financial impact. *J Pediatr Surg* 1998;**33**:830-3.
25 Yanchar NL, Soucy P. Long-term outcome after Hirschsprung's disease: patients' perspectives. *J Pediatr Surg* 1999;**34**:1152-60.
26 Moore SW, Albertyn R, Cywes S. Clinical outcome and long-term quality of life after surgical correction of Hirschsprung's disease. *J Pediatr Surg* 1996;**31**:1496-502.
27 Albanese CT, Jennings RW, Smith B, Bratton B, Harrison MR. Perineal one-stage pull-through for Hirschsprung's disease. *J Pediatr Surg* 1999;**34**:377-80.
28 Jasonni V, Martucciello G. Total colonic aganglionosis. *Semin Pediatr Surg* 1998;**7**:174-80.
29 Tsuji H, Spitz L, Kiely EM, Drake DP, Pierro A. Management and long-term follow-up of infants with total colonic aganglionosis. *J Pediatr Surg* 1999;**34**:158-62.
30 Puffenberger EG, Kauffman ER, Bolk S, Matise TC, Washington SS, Angrist M, Weissenbach J, Garver KL, Mascari M, Ladda R, Slaugenhaupt SA, Chakravarti A. Identity-by-descent and association mapping of a recessive gene for Hirschsprung disease on human chromosome 13q22. *Hum Mol Genet* 1994;**3**:1217-25.
31 Salomon R, Attie T, Pelet A, Bidaud C, Eng C, Amiel J, Sarnacki S, Goulet O, Ricour C, Nihoul-Fekete C, Munnich A, Lyonnet S. Germline mutations of the RET ligand GDNF are not sufficient to cause Hirschsprung disease. *Nat Genet* 1996;**14**:345-7.
32 Sakai T, Wakizaka A, Nirasawa Y, Ito Y. Point nucleotidic changes in both the RET proto-oncogene and the endothelin-B receptor gene in a Hirschsprung disease patient associated with Down syndrome. *Tohoku J Exp Med* 1999;**187**:43-7.
33 Martucciello G, Biocchini M, Dodero P, Cirillo M, Puliti A, Gimelli G. Total colonic aganglionosis associated with interstitial deletion of the long arm of chromosome 10. *Pediatr Surg Int* 1992;**7**:308.

34 Fewtrell MS, Tam PK, Thomson AH, Fitchett M, Currie J, Huson SM, Mulligan LM. Hirschsprung's disease associated with a deletion of chromosome 10 (q11.2q21.2): a further link with the neurocristopathies? *J Med Genet* 1994; **31**:325-7.

35 Sparkes RS, Sparkes MC, Kalina RE, Pagon RA, Salk DJ, Disteche CM. Separation of retinoblastoma and esterase D loci in a patient with sporadic retinoblastoma and del(13)(q14.1q22.3). *Hum Genet* 1984;**68**:258-9.

36 Lamont MA, Fitchett M, Dennis NR. Interstitial deletion of distal 13q associated with Hirschsprung's disease. *J Med Genet* 1989;**26**:100-4.

37 Bottani A, Xie YG, Binkert F, Schinzel A. A case of Hirschsprung disease with a chromosome 13 microdeletion, del(13)(q32.3q33.2): potential mapping of one disease locus. *Hum Genet* 1991;**87**:748-50.

38 Lurie IW, Supovitz KR, Rosenblum-Vos LS, Wulfsberg EA. Phenotypic variability of del(2) (q22-q23): report of a case with a review of the literature. *Genet Couns* 1994;**5**:11-14.

39 Mowat DR, Croaker GD, Cass DT, Kerr BA, Chaitow J, Ades LC, Chia NL, Wilson MJ. Hirschsprung disease, microcephaly, mental retardation, and characteristic facial features: delineation of a new syndrome and identification of a locus at chromosome 2q22-q23. *J Med Genet* 1998;**35**:617-23.

40 McMilin KD, Reiss JA, Brown MG, Black MH, Buckmaster DA, Durum CT, Gunter KA, Lawce HJ, Berry TL, Lamb OA, Olson CL, Weeks FF, Yoshitomi MJ, Jacky PB, Olson SB, Magenis RE. Clinical outcomes of four patients with microdeletion in the long arm of chromosome 2. *Am J Med Genet* 1998;**78**:36-43.

41 Wakamatsu N, Yamada Y, Yamada K, On T, Nomura N, Taniguchi H, Kitoh H, Mutoh N, Yamanaka T, Mushiake K, Kato K, Sonta S, Nagaya M. Mutations in SIP1, encoding Smad interacting protein-1, cause a form of Hirschsprung disease. *Nat Genet* 2001;**27**:369-70.

42 Hansford JR, Mulligan LM. Multiple endocrine neoplasia type 2 and RET: from neoplasia to neurogenesis. *J Med Genet* 2000;**37**:817-27.

43 Verdy M, Weber AM, Roy CC, Morin CL, Cadotte M, Brochu P. Hirschsprung's disease in a family with multiple endocrine neoplasia type 2. *J Pediatr Gastroenterol Nutr* 1982;**1**:603-7.

44 Mulligan LM, Eng C, Attie T, Lyonnet S, Marsh DJ, Hyland VJ, Robinson BG, Frilling A, Verellen-Dumoulin C, Safar A, Venter DJ, Munnich A, Ponder BAJ. Diverse phenotypes associated with exon 10 mutations of the RET proto-oncogene. *Hum Mol Genet* 1994;**3**:2163-7.

45 Borst MJ, VanCamp JM, Peacock ML, Decker RA. Mutational analysis of multiple endocrine neoplasia type 2A associated with Hirschsprung's disease. *Surgery* 1995; **117**:386-91.

46 Caron P, Attie T, David D, Amiel J, Brousset F, Roger P, Munnich A, Lyonnet S. C618R mutation in exon 10 of the RET proto-oncogene in a kindred with multiple endocrine neoplasia type 2A and Hirschsprung's disease. *J Clin Endocrinol Metab* 1996;**81**:2731-3.

47 Peretz H, Luboshitsky R, Baron E, Biton A, Gershoni R, Usher S, Grynberg E, Yakobson E, Graff E, Lapidot M. Cys 618 Arg mutation in the RET proto-oncogene associated with familial medullary thyroid carcinoma and maternally transmitted Hirschsprung's disease suggesting a role for imprinting. *Hum Mutat* 1997;**10**:155-9.

48 Decker RA, Peacock ML, Watson P. Hirschsprung disease in MEN 2A: increased spectrum of RET exon 10 genotypes and strong genotype-phenotype correlation. *Hum Mol Genet* 1998;**7**:129-34.

49 Romeo G, Ceccherini I, Celli J, Priolo M, Betsos N, Bonardi G, Seri M, Yin L, Lerone M, Jasonni V, Martucciello G. Association of multiple endocrine neoplasia type 2 and Hirschsprung disease. *J Intern Med* 1998;**243**:515-20.

50 Borrego S, Eng C, Sanchez B, Saez ME, Navarro E, Antinolo G. Molecular analysis of the ret and GDNF genes in a family with multiple endocrine neoplasia type 2A and Hirschsprung disease. *J Clin Endocrinol Metab* 1998;**83**: 3361-4.

51 Waardenburg PJ. A new syndrome combining developmental anomalies of the eyelids, eyebrows and nose root with pigmentary defects of the iris and head hair and with congenital deafness. *Am J Hum Genet* 1951;**3**:195-253.

52 Hageman MJD. Heterogeneity in Waardenburg syndrome. *Am J Hum Genet* 1977;**29**:468-85.

53 Puffenberger EG, Hosoda K, Washington SS, Nakao K, deWit D, Yanagisawa M, Chakravarti A. A missense mutation of the endothelin-B receptor gene in multigenic Hirschsprung's disease. *Cell* 1994;**79**:1257-66.

54 Attie T, Till M, Pelet A, Amiel J, Edery P, Boutrand L, Munnich A, Lyonnet S. Mutation of the endothelin-receptor B gene in Waardenburg-Hirschsprung disease. *Hum Mol Genet* 1995;**4**:2407-9.

55 Hofstra RM, Osinga J, Tan-Sindhunata G, Wu Y, Kamsteeg EJ, Stulp RP, van Ravenswaaij-Arts C, Majoor-Krakauer D, Angrist M, Chakravarti A, Meijers C, Buys CH. A homozygous mutation in the endothelin-3 gene associated with a combined Waardenburg type 2 and Hirschsprung phenotype (Shah-Waardenburg syndrome). *Nat Genet* 1996;**12**:445-7.

56 Edery P, Attie T, Amiel J, Pelet A, Eng C, Hofstra RM, Martelli H, Bidaud C, Munnich A, Lyonnet S. Mutation of the endothelin-3 gene in the Waardenburg-Hirschsprung disease (Shah-Waardenburg syndrome). *Nat Genet* 1996; **12**:442-4.

57 Pingault V, Bondurand N, Kuhlbrodt K, Goerich DE, Prehu MO, Puliti A, Herbarth B, Hermans-Borgmeyer I, Legius E, Matthijs G, Amiel J, Lyonnet S, Ceccherini I, Romeo G, Smith JC, Read AP, Wegner M, Goossens M. SOX10 mutations in patients with Waardenburg-Hirschsprung disease. *Nat Genet* 1998;**18**:171-3.

58 Jacobs JM, Wilson J. An unusual demyelinating neuropathy in a patient with Waardenburg's syndrome. *Acta Neuropathol (Berl)* 1992;**83**:670-4.

59 Touraine RL, Attie-Bitach T, Manceau E, Korsch E, Sarda P, Pingault V, Encha-Razavi F, Pelet A, Auge J, Nivelon-Chevallier A, Holschneider AM, Munnes M, Doerfler W, Goossens M, Munnich A, Vekemans M, Lyonnet S. Neurological phenotype in Waardenburg syndrome type 4 correlates with novel SOX10 truncating mutations and expression in developing brain. *Am J Hum Genet* 2000;**66**: 1496-503.

60 Bondurand N, Kuhlbrodt K, Pingault V, Enderich J, Sajus M, Tommerup N, Warburg M, Hennekam RC, Read AP, Wegner M, Goossens M. A molecular analysis of the Yemenite deaf-blind hypopigmentation syndrome: SOX10 dysfunction causes different neurocristopathies. *Hum Mol Genet* 1999;**8**:1785-9.

61 Gross A, Kunze J, Maier RF, Stoltenburg-Didinger G, Grimmer I, Obladen M. Autosomal-recessive neural crest syndrome with albinism, black lock, cell migration disorder of the neurocytes of the gut, and deafness: ABCD syndrome. *Am J Med Genet* 1995;**56**:322-6.

62 Mahakrishnan A, Srinivasan MS. Piebaldness with Hirschsprung's disease. *Arch Dermatol* 1980;**116**:1102.

63 Kaplan P, de Chaderevian JP. Piebaldism-Waardenburg syndrome: histopathologic evidence for a neural crest syndrome. *Am J Med Genet* 1988;**31**:679-88.

64 Weinberg AG, Currarino G, Besserman AM. Hirschsprung's disease and congenital deafness. Familial association. *Hum Genet* 1977;**38**:157-61.

65 Gozal D. Congenital central hypoventilation syndrome: an update. *Pediatr Pulmonol* 1998;**26**:273-82.

66 Chatten J, Voorhess ML. Familial neuroblastoma. Report of a kindred with multiple disorders, including neuroblastomas in four siblings. *N Engl J Med* 1967;**277**:1230-6.

67 Roshkow JE, Haller JO, Berdon WE, Sane SM. Hirschsprung's disease, Ondine's curse, and neuroblastoma - manifestations of neurocristopathy. *Pediatr Radiol* 1988;**19**: 45-9.

68 Levard G, Boige N, Vitoux C, Aigrain Y, Boureau M, Navarro J. Neurocristopathy. The association of Hirschsprung's disease-ganglioneuroma with autonomic nervous system dysfunction in 2 children. *Arch Fr Pediatr* 1989;**46**:595-7.

69 Weese-Mayer DE, Silvestri JM, Marazita ML, Hoo JJ. Congenital central hypoventilation syndrome: inheritance and relation to sudden infant death syndrome. *Am J Med Genet* 1993;**47**:360-7.

70 Haddad GG, Mazza NM, Defendini R, Blanc WA, Driscoll JM, Epstein MA, Epstein RA, Mellins RB. Congenital failure of automatic control of ventilation, gastrointestinal motility and heart rate. *Medicine* 1978;**57**:517-26.

71 Verloes A, Elmer C, Lacombe D, Heinrichs C, Rebuffat E, Demarquez JL, Moncla A, Adam E. Ondine-Hirschsprung syndrome (Haddad syndrome). Further delineation in two cases and review of the literature. *Eur J Pediatr* 1993;**152**:75-7.

72 Amiel J, Salomon R, Attie T, Pelet A, Trang H, Mokhtari M, Gaultier C, Munnich A, Lyonnet S. Mutations of the RET-GDNF signaling pathway in Ondine's curse. *Am J Hum Genet* 1996;**62**:715-17.

73 Sakai T, Wakizaka A, Matsuda H, Nirasawa Y, Itoh Y. Point mutation in exon 12 of the receptor tyrosine kinase proto-oncogene RET in Ondine-Hirschsprung syndrome. *Pediatrics* 1998;**101**:924-6.

74 Bolk S, Angrist M, Xie J, Yanagisawa M, Silvestri JM, Weese-Mayer DE, Chakravarti A. Endothelin-3 frameshift mutation in congenital central hypoventilation syndrome. *Nat Genet* 1996;**13**:395-6.

75 Gaisie G, Oh KS, Young LW. Coexistent neuroblastoma and Hirschsprung's disease -another manifestation of the neurocristopathy? *Pediatr Radiol* 1979;**8**:161-3.

76 Michna BA, McWilliams NB, Krummel TM, Hartenberg MA, Salzberg AM. Multifocal ganglioneuroblastoma coexistent with total colonic aganglionosis. *J Pediatr Surg* 1988; **23**:57-9.

77 Merkler RG, Solish SB, Scherzer AL. Meningomyelocele and Hirschprung disease: theoretical and clinical significance. *Pediatrics* 1985;**76**:299-300.

78 Clausen N, Andersson P, Tommerup N. Familial occurrence of neuroblastoma, von Recklinghausen's neurofibromatosis, Hirschsprung's agangliosis and jaw-winking syndrome. *Acta Paediatr Scand* 1989;**78**:736-41.

79 Goldberg RB, Shprintzen RJ. Hirschsprung megacolon and cleft palate in two sibs. *J Craniofac Genet Dev Biol* 1981;**1**:185-9.

80 Hurst JA, Markiewicz M, Kumar D, Brett EM. Unknown syndrome: Hirschsprung's disease, microcephaly, and iris coloboma: a new syndrome of defective neuronal migration. *J Med Genet* 1988;**25**:494-7.

81 Halal F, Morel J. The syndrome of Hirschsprung disease, microcephaly, unusual face, and mental retardation. *Am J Med Genet* 1990;**37**:106-8.

82 Santos H, Mateus J, Leal MJ. Hirschsprung disease associated with polydactyly, unilateral renal agenesis, hypertelorism, and congenital deafness: a new autosomal recessive syndrome. *J Med Genet* 1988;**25**:204-5.

83 Laurence KM, Prosser R, Rocker I, Pearson JF, Richard C. Hirschsprung's disease associated with congenital heart malformation, broad big toes, and ulnar polydactyly in sibs: a case for fetoscopy. *J Med Genet* 1975;**12**:334-8.

84 Al-Gazali LI, Donnai D, Mueller RF. Hirschsprung's disease, hypoplastic nails, and minor dysmorphic features: a distinct autosomal recessive syndrome? *J Med Genet* 1988;**25**:758-61.

85 Huang T, Elias ER, Mulliken JB, Kirse DJ, Holmes LB. A new syndrome: heart defect, laryngeal anomalies, preaxial polydactyly, and colonic aganglionosis in sibs. *Genet Med* 1999;**1**:104.

86 Reynolds JF, Barber JC, Alford BA, Chandler JG, Kelly TE. Familial Hirschsprung's disease and type D brachydactyly: a report of four affected males in two generations. *Pediatrics* 1983;**71**:246-9.

87 Toriello HV, Komar K, Lawrence C, Higgins JV, Waterman DF. Macrocephaly, Hirschsprung disease, brachydactyly, vertebral defects, and other minor anomalies. *Dysmorphol Clin Genet* 1988;**1**:155-7.

88 Reish O, Gorlin RJ, Hordinsky M, Rest EB, Burke B, Berry SA. Brain anomalies, retardation of mentality and growth, ectodermal dysplasia, skeletal malformations, Hirschsprung disease, ear deformity and deafness, eye hypoplasia, cleft palate, cryptorchidism, and kidney dysplasia/hypoplasia (BRESEK/BRESHECK): new X-linked syndrome? *Am J Med Genet* 1997;**68**:386-90.

89 Davenport M, Taitz LS, Dickson JA. The Kaufman-McKusick syndrome: another association. *J Pediatr Surg* 1989;**24**:1192-4.

90 Stone DL, Slavotinek A, Bouffard GG, Banerjee-Basu S, Baxevanis AD, Barr M, Biesecker LG. Mutation of a gene encoding a putative chaperonin causes McKusick-Kaufman syndrome. *Nat Genet* 2000;**25**:79-82.

91 Radetti G, Frick R, Pasquino B, Mengarda G, Savage MO. Hypothalamic-pituitary dysfunction and Hirschsprung's disease in the Bardet-Biedl syndrome. *Helv Paediatr Acta* 1988;**43**:249-52.

92 Lorda-Sanchez I, Ayuso C, Ibanez A. Situs inversus and Hirschsprung disease: two uncommon manifestations in Bardet-Biedl syndrome. *Am J Med Genet* 2000;**90**:80-1.

93 Slavotinek AM, Stone EM, Mykytyn K, Heckenlively JR, Green JS, Heon E, Musarella MA, Parfrey PS, Sheffield VC, Biesecker LG. Mutations in MKKS cause Bardet-Biedl syndrome. *Nat Genet* 2000;**26**:15-16.

94 Wassif CA, Maslen C, Kachilele-Linjewile S, Lin D, Linck LM, Connor WE, Steiner RD, Porter FD. Mutations in the human sterol delta7-reductase gene at 11q12-13 cause Smith-Lemli-Opitz syndrome. *Am J Hum Genet* 1998;**63**:55-62.

95 Waterham HR, Wijburg FA, Hennekam RC, Vreken P, Poll-The BT, Dorland L, Duran M, Jira PE, Smeitink JA, Wevers RA, Wanders RJ. Smith-Lemli-Opitz syndrome is caused by mutations in the 7-dehydrocholesterol reductase gene. *Am J Hum Genet* 1998;**63**:329-38.

96 Patterson K, Toomey KE, Chandra RS. Hirschsprung disease in a 46,XY phenotypic infant girl with Smith-Lemli-Opitz syndrome. *J Pediatr* 1983;**103**:425-7.

97 Makitie O, Kaitila I. Cartilage-hair hypoplasia - clinical manifestations in 108 Finnish patients. *Eur J Pediatr* 1993;**152**:211-17.

98 Sulisalo T, Sistonen P, Hastbacka J, Wadelius C, Makitie O, de la Chapelle A, Kaitila I. Cartilage-hair hypoplasia gene assigned to chromosome 9 by linkage analysis. *Nat Genet* 1993;**3**:338-41.

99 Mandel H, Brik R, Ludatscher R, Braun J, Berant M. Congenital muscular dystrophy with neurological abnormalities: association with Hirschsprung disease. *Am J Med Genet* 1993;**47**:37-40.

100 Kim JJ, Armstrong DD, Fishman MA. Multicore myopathy, microcephaly, and aganglionosis, and short stature. *J Child Neurol* 1994;**9**:275-7.

101 Mallory SB, Haynie LS, Williams ML, Hall W. Ichthyosis, deafness, and Hirschsprung's disease. *Pediatr Dermatol* 1989;**6**:24-7.

102 Kaplan P. X linked recessive inheritance of agenesis of the corpus callosum. *J Med Genet* 1983;**20**:122-4.

103 Okamoto N, Wada Y, Goto M. Hydrocephalus and Hirschsprung's disease in a patient with a mutation of L1CAM. *J Med Genet* 1997;**34**:670-1.

104 Ikeda K, Goto S. Additional anomalies in Hirschsprung's disease: an analysis based on the nationwide survey in Japan. *Z Kinderchir* 1986;**41**:279-81.

105 Edward E, Ecker J, Christakis N, Folkman J. Hirschsprung's disease: associated abnormalities and demography. *J Pediatr Surg* 1992;**27**:76.

106 Russell MB, Russell CA, Niebuhr E. An epidemiological study of Hirschsprung's disease and additional anomalies. *Acta Paediatr* 1994;**83**:68-71.

107 Sarioglu A, Tanyel FC, Buyukpamukcu N, Hicsonmez A. Hirschsprung-associated congenital anomalies. *Eur J Pediatr Surg* 1997;**7**:331-7.

108 Schuchardt A, D'Agati V, Larsson-Blomberg L, Costantini F, Pachnis V. Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor Ret. *Nature* 1994;**367**:380-3.

109 Whalen TV Jr, Asch MJ. Report of two patients with hypertrophic pyloric stenosis and Hirschsprung's disease. Coincident or common etiology? *Am Surg* 1985;**51**:480-1.

110 Sayed M, al-Alaiyan S. Agenesis of corpus callosum, hypertrophic pyloric stenosis and Hirschsprung disease: coincidence or common etiology? *Neuropediatrics* 1996;**27**:204-6.

111 Janik JP, Wayne ER, Janik JS, Price MR. Ileal atresia with total colonic aganglionosis. *J Pediatr Surg* 1997;**32**:1502-3.

112 Lyonnet S, Bolino A, Pelet A, Abel L, Nihoul-Fekete C, Briard ML, Mok-Siu V, Kaariainen H, Martucciello G, Lerone M, Puliti A, Luo Y, Weissenbach J, Devoto M, Munnich A, Romeo G. A gene for Hirschsprung disease maps to the proximal long arm of chromosome 10. *Nat Genet* 1993;**4**:346-50.

113 Angrist M, Kauffman E, Slaugenhaupt SA, Matise TC, Puffenberger EG, Washington SS, Lipson A, Cass DT, Reyna T, Weeks DE, Sieber W, Chakravati A. A gene for Hirschsprung disease (megacolon) in the pericentromeric region of human chromosome 10. *Nat Genet* 1993;**4**:351-6.

114 Mulligan LM, Kwok JB, Healey CS, Elsdon MJ, Eng C, Gardner E, Love DR, Mole SE, Moore JK, Papi L. Germline mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. *Nature* 1993;**363**:458-60.

115 Donis-Keller H, Dou S, Chi D, Carlson KM, Toshima K, Lairmore TC, Howe JR, Moley JF, Goodfellow P, Wells SA. Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. *Hum Mol Genet* 1993;**2**:851-6.

116 Edery P, Lyonnet S, Mulligan LM, Pelet A, Dow E, Abel L, Holder S, Nihoul-Fekete C, Ponder BA, Munnich A. Mutations of the RET proto-oncogene in Hirschsprung's disease. *Nature* 1994;**367**:378-80.

117 Romeo G, Ronchetto P, Luo Y, Barone V, Seri M, Ceccherini I, Pasini B, Bocciardi R, Lerone M, Kaariainen H, Martucciello G. Point mutations affecting the tyrosine kinase domain of the RET proto-oncogene in Hirschsprung's disease. *Nature* 1994;**367**:377-8.

118 Ceccherini I, Bocciardi R, Luo Y, Pasini B, Hofstra R, Takahashi M, Romeo G. Exon structure and flanking intronic sequences of the human RET proto-oncogene. *Biochem Biophys Res Commun* 1993;**196**:1288-95.

119 Attie T, Pelet A, Edery P, Eng C, Mulligan LM, Amiel J, Boutrand L, Beldjord C, Nihoul-Fekete C, Munnich A, Ponder BAJ, Lyonnet S. Diversity of RET proto-oncogene mutations in familial and sporadic Hirschsprung disease. *Hum Mol Genet* 1995;**4**:1381-6.

120 Angrist M, Bolk S, Thiel B, Puffenberger EG, Hofstra RM, Buys CH, Cass DT, Chakravarti A. Mutation analysis of the RET receptor tyrosine kinase in Hirschsprung disease. *Hum Mol Genet* 1995;**4**:821-30.

121 Seri M, Yin L, Barone V, Bolino A, Celli I, Bocciardi R, Pasini B, Ceccherini I, Lerone M, Kristoffersson U, Larsson LT, Casasa JM, Cass DT, Abramowicz MJ, Vanderwinden JM, Kravcenkiene I, Baric I, Silengo M, Martucciello G, Romeo G. Frequency of RET mutations in long- and short-segment Hirschsprung disease. *Hum Mutat* 1997;**9**:243-9.

122 Eng C, Smith DP, Mulligan LM, Nagai MA, Healey CS, Ponder MA, Gardner E, Scheumann GF, Jackson CE, Tunnacliffe A, Ponder BAJ. Point mutation within the tyrosine kinase domain of the RET proto-oncogene in multiple endocrine neoplasia type 2B and related sporadic tumours [published erratum appears in *Hum Mol Genet* 1994;**3**:686]. *Hum Mol Genet* 1994;**3**:237-41.

123 Hofstra RM, Landsvater RM, Ceccherini I, Stulp R, Stelwagen T, Luo Y, Pasini B, Hoppener JW, van Amstel HKGR. A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. *Nature* 1994;**367**:375-6.

124 Carlson KM, Dou S, Chi D, Scavarda N, Toshima K, Jackson CE, Wells SAJ, Goodfellow PJ, Donis-Keller H. Single missense mutation in the tyrosine kinase catalytic domain of the RET protooncogene is associated with multiple endocrine neoplasia type 2B. *Proc Natl Acad Sci USA* 1994;**91**:1579-83.

125 Santoro M, Carlomagno F, Romano A, Bottaro DP, Dathan NA, Grieco M, Fusco A, Vecchio G, Matoskova B, Kraus MH. Activation of RET as a dominant transforming gene by germline mutations of MEN2A and MEN2B. *Science* 1995;**267**:381-3.

126 Pasini B, Borrello MG, Greco A, Bongarzone I, Luo Y, Mondellini P, Alberti L, Miranda C, Arighi E, Bocciardi R, Seri M, Barone V, Radice MT, Romeo G, Pierotti MA. Loss of function effect of RET mutations causing Hirschsprung disease. *Nat Genet* 1995;**10**:35-40.

127 Carlomagno F, De Vita G, Berlingieri MT, de Franciscis V, Melillo RM, Colantuoni V, Kraus MH, Di Fiore PP, Fusco A, Santoro M. Molecular heterogeneity of RET loss of function in Hirschsprung's disease. *EMBO J* 1996;**15**:2717-25.

128 Pelet A, Geneste O, Edery P, Pasini A, Chappuis S, Atti T, Munnich A, Lenoir G, Lyonnet S, Billaud M. Various mechanisms cause RET-mediated signaling defects in Hirschsprung's disease. *J Clin Invest* 1998;**101**:1415-23.

129 Bolk S, Pelet A, Hofstra RM, Angrist M, Salomon R, Croaker D, Buys CH, Lyonnet S, Chakravarti A. A human model for multigenic inheritance: phenotypic expression in Hirschsprung disease requires both the RET gene and a new 9q31 locus. *Proc Natl Acad Sci USA* 2000;**97**:268-73.

130 Borrego S, Saez ME, Ruiz A, Gimm O, Lopez-Alonso M, Antinolo G, Eng C. Specific polymorphisms in the RET proto-oncogene are over-represented in patients with Hirschsprung disease and may represent loci modifying phenotypic expression. *J Med Genet* 1999;**36**:771-4.

131 Borrego S, Ruiz A, Saez ME, Gimm O, Gao X, Lopez-Alonso M, Hernandez A, Wright FA, Antinolo G, Eng C. RET genotypes comprising specific haplotypes of polymorphic variants predispose to isolated Hirschsprung disease. *J Med Genet* 2000;**37**:572-8.

132 Moore MW, Klein RD, Farinas I, Sauer H, Armanini M, Phillips H, Reichardt LF, Ryan AM, Carver-Moore K, Rosenthal A. Renal and neuronal abnormalities in mice lacking GDNF. *Nature* 1996;**382**:76-9.

133 Sanchez MP, Silos-Santiago I, Frisen J, He B, Lira SA, Barbacid M. Renal agenesis and the absence of enteric neurons in mice lacking GDNF. *Nature* 1996;**382**:70-3.

134 Pichel JG, Shen L, Sheng HZ, Granholm AC, Drago J, Grinberg A, Lee EJ, Huang SP, Saarma M, Hoffer BJ, Sariola H, Westphal H. Defects in enteric innervation and kidney development in mice lacking GDNF. *Nature* 1996;**382**: 73-6.

135 Durbec P, Marcos-Gutierrez CV, Kilkenny C, Grigoriou M, Wartiowaara K, Suvanto P, Smith D, Ponder B, Costantini F, Saarma M, Sariola H, Pachnis V. GDNF signalling through the Ret receptor tyrosine kinase. *Nature* 1996;**381**: 789-93.

136 Jing S, Wen D, Yu Y, Holst PL, Luo Y, Fang M, Tamir R, Antonio L, Hu Z, Cupples R, Louis JC, Hu S, Altrock BW, Fox GM. GDNF-induced activation of the ret protein tyrosine kinase is mediated by GDNFR-alpha, a novel receptor for GDNF. *Cell* 1996;**85**:1113-24.

137 Treanor JJ, Goodman L, de Sauvage F, Stone DM, Poulsen KT, Beck CD, Gray C, Armanini MP, Pollock RA, Hefti F, Phillips HS, Goddard A, Moore MW, Buj-Bello A, Davies AM, Asai N, Takahashi M, Vandlen R, Henderson CE, Rosenthal A. Characterization of a multicomponent receptor for GDNF. *Nature* 1996;**382**:80-3.

138 Rosenthal A. The GDNF protein family: gene ablation studies reveal what they really do and how. *Neuron* 1999;**22**:201-3.

139 Kotzbauer PT, Lampe PA, Heuckeroth RO, Golden JP, Creedon DJ, Johnson EM, Milbrandt J. Neurturin, a relative of glial-cell-line-derived neurotrophic factor. *Nature* 1996;**384**:467-70.

140 Milbrandt J, de Sauvage FJ, Fahrner TJ, Baloh RH, Leitner ML, Tansey MG, Lampe PA, Heuckeroth RO, Kotzbauer PT, Simburger KS, Golden JP, Davies JA, Vejsada R, Kato AC, Hynes M, Sherman D, Nishimura M, Wang LC, Vandlen R, Moffat B, Klein RD, Poulsen K, Gray C, Garces A, Johnson EM, Henderson CE, Phillips HS, Johnson EM Jr. Persephin, a novel neurotrophic factor related to GDNF and neurturin. *Neuron* 1998;**20**:245-53.

141 Baloh RH, Tansey MG, Lampe PA, Fahrner TJ, Enomoto H, Simburger KS, Leitner ML, Araki T, Johnson EM, Milbrandt J. Artemin, a novel member of the GDNF ligand family, supports peripheral and central neurons and signals through the GFRalpha3-RET receptor complex. *Neuron* 1998;**21**:1291-302.

142 Angrist M, Bolk S, Halushka M, Lapchak PA, Chakravarti A. Germline mutations in glial cell line-derived neurotrophic factor (GDNF) and RET in a Hirschsprung disease patient. *Nat Genet* 1996;**14**:341-4.

143 Ivanchuk SM, Myers SM, Eng C, Mulligan LM. De novo mutation of GDNF, ligand for the RET/GDNFR-alpha receptor complex, in Hirschsprung disease. *Hum Mol Genet* 1996;**5**:2023-6.

144 Doray B, Salomon R, Amiel J, Pelet A, Touraine R, Billaud M, Attie T, Bachy B, Munnich A, Lyonnet S. Mutation of the RET ligand, neurturin, supports multigenic inheritance in Hirschsprung disease. *Hum Mol Genet* 1998;**7**:1449-52.

145 Cacalano G, Farinas I, Wang LC, Hagler K, Forgie A, Moore M, Armanini M, Phillips H, Ryan AM, Reichardt LF, Hynes M, Davies A, Rosenthal A. GFRalpha1 is an essential receptor component for GDNF in the developing nervous system and kidney. *Neuron* 1998;**21**:53-62.

146 Enomoto H, Araki T, Jackman A, Heuckeroth RO, Snider WD, Johnson EM, Milbrandt J. GFR alpha1-deficient mice have deficits in the enteric nervous system and kidneys. *Neuron* 1998;**21**:317-24.

147 Angrist M, Jing S, Bolk S, Bentley K, Nallasamy S, Halushka M, Fox GM, Chakravarti A. Human GFRA1: cloning, mapping, genomic structure, and evaluation as a candidate gene for Hirschsprung disease susceptibility. *Genomics* 1998;**48**:354-62.

148 Myers SM, Salomon R, Goessling A, Pelet A, Eng C, von Deimling A, Lyonnet S, Mulligan LM. Investigation of germline GFRα-1 mutations in Hirschsprung disease. *J Med Genet* 1999;**36**:217-20.

149 Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyauchi T, Goto K, Masaki T. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc Natl Acad Sci USA* 1989;**86**:2863-7.

150 Sakurai T, Yanagisawa M, Masaki T. Molecular characterization of endothelin receptors. *Trends Pharmacol Sci* 1992; **13**:103-8.

151 Cohen IT, Gadd MA. Hirschsprung's disease in a kindred: a possible clue to the genetics of the disease. *J Pediatr Surg* 1982;**17**:632-4.

152 Van Camp G, Van Thienen MN, Handig I, Van Roy B, Rao VS, Milunsky A, Read AP, Baldwin CT, Farrer LA, Bonduelle M, Standaert L, Meire F, Willems PJ. Chromosome 13q deletion with Waardenburg syndrome: further evidence for a gene involved in neural crest function on 13q. *J Med Genet* 1995;**32**:531-6.

153 Kiss P, Orsztovics M. Association of 13q deletion and Hirschsprung's disease. *J Med Genet* 1989;**26**:793-4.

154 Hosoda K, Hammer RE, Richardson JA, Baynash AG, Cheung JC, Giaid A, Yanagisawa M. Targeted and natural (piebald-lethal) mutations of endothelin-B receptor gene produce megacolon associated with spotted coat color in mice. *Cell* 1994;**79**:1267-76.

155 Baynash AG, Hosoda K, Giaid A, Richardson JA, Emoto N, Hammer RE, Yanagisawa M. Interaction of endothelin-3 with endothelin-B receptor is essential for development of epidermal melanocytes and enteric neurons. *Cell* 1994;**79**:1277-85.

156 Bidaud C, Salomon R, Van Camp G, Pelet A, Attie T, Eng C, Bonduelle M, Amiel J, Nihoul-Fekete C, Willems PJ, Munnich A, Lyonnet S. Endothelin-3 gene mutations in isolated and syndromic Hirschsprung disease. *Eur J Hum Genet* 1997;**5**:247-51.

157 Chakravarti A. Endothelin receptor-mediated signaling in Hirschsprung disease. *Hum Mol Genet* 1996;**5**:303-7.

158 Amiel J, Attie T, Jan D, Pelet A, Edery P, Bidaud C, Lacombe D, Tam P, Simeoni J, Flori E, Nihoul-Fekete C, Munnich A, Lyonnet S. Heterozygous endothelin receptor B (EDNRB) mutations in isolated Hirschsprung disease. *Hum Mol Genet* 1996;**5**:355-7.

159 Kusafuka T, Wang Y, Puri P. Novel mutations of the endothelin-B receptor gene in isolated patients with Hirschsprung's disease. *Hum Mol Genet* 1996;**5**:347-9.

160 Auricchio A, Casari G, Staiano A, Ballabio A. Endothelin-B receptor mutations in patients with isolated Hirschsprung disease from a non-inbred population. *Hum Mol Genet* 1996;**5**:351-4.

161 Yanagisawa H, Yanagisawa M, Kapur RP, Richardson JA, Williams SC, Clouthier DE, de Wit D, Emoto N, Hammer RE. Dual genetic pathways of endothelin-mediated intercellular signaling revealed by targeted disruption of endothelin converting enzyme-1 gene. *Development* 1998; **125**:825-36.

162 Hofstra RM, Valdenaire O, Arch E, Osinga J, Kroes H, Loffler BM, Hamosh A, Meijers C, Buys CH. A loss-of-function mutation in the endothelin-converting enzyme 1 (ECE-1) associated with Hirschsprung disease, cardiac defects, and autonomic dysfunction. *Am J Hum Genet* 1999;**64**:304-8.

163 Lane PW, Liu HM. Association of megacolon with a new dominant spotting gene (Dom) in the mouse. *J Hered* 1984;**75**:435-9.

164 Herbarth B, Pingault V, Bondurand N, Kuhlbrodt K, Hermans-Borgmeyer I, Puliti A, Lemort N, Goossens M, Wegner M. Mutation of the Sry-related Sox10 gene in dominant megacolon, a mouse model for human Hirschsprung disease. *Proc Natl Acad Sci USA* 1998;**95**:5161-5.

165 Southard-Smith EM, Angrist M, Ellison JS, Agarwala R, Baxevanis AD, Chakravarti A, Pavan WJ. The Sox10 (Dom) mouse: modeling the genetic variation of Waardenburg-Shah (WS4) syndrome. *Genome Res* 1999;**9**: 215-25.

166 Auricchio A, Griseri P, Carpentieri ML, Betsos N, Staiano A, Tozzi A, Priolo M, Thompson H, Bocciardi R, Romeo G, Ballabio A, Ceccherini I. Double heterozygosity for a RET substitution interfering with splicing and an EDNRB missense mutation in Hirschsprung disease. *Am J Hum Genet* 1999;**64**:1216-21.

167 Potterf SB, Furumura M, Dunn KJ, Arnheiter H, Pavan WJ. Transcription factor hierarchy in Waardenburg syndrome: regulation of MITF expression by SOX10 and PAX3. *Hum Genet* 2000;**107**:1-6.

168 Southard-Smith EM, Kos L, Pavan WJ. Sox10 mutation disrupts neural crest development in Dom Hirschsprung mouse model. *Nat Genet* 1998;**18**:60-4.

169 Bolk S, Angrist M, Fornage M, Olson JM, Hofstra R, Buys C, Chakravarti A (group I). Salomon R, Pelet A, Amiel J, Attié-Bitach T, Steffann J, Munnich A, Lyonnet S (group II). Splitting a multigenic disease: segregation at three loci explains familial and population risk in Hirschsprung disease (in preparation).

170 Hall CM. Werner's mesomelic dysplasia with ventricular septal defect and Hirschsprung's disease. *Pediatr Radiol* 1981;**10**:247–9.