

A Hirschsprung disease locus at 22q11?

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Abstract

We report a boy with truncus arteriosus, dysmorphic features, developmental delay, passing hypotonia, short segment Hirschsprung disease (HSCR), and paroxysmal hypoventilation. FISH analysis showed an interstitial deletion in chromosome band 22q11.2 coinciding with the deletions found in DiGeorge syndrome and velocardiofacial syndrome. Mutation scanning of RET, GDNF, EDNRB, and EDN3, genes associated with Hirschsprung disease, showed no aberrations. Since we know of two more patients with velocardiofacial syndrome and HSCR, we hypothesise that a gene responsible for proper development of the enteric nervous system may be included in the 22q11.2 region.

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Deletions of the chromosomal region 22q11.2 are associated with a wide spectrum of congenital malformations, affecting the face, palate, parathyroid glands, thymus, heart, limbs, and kidneys. Several distinct clinical syndromes have been recognised to be associated with 22q11.2 deletions, including DiGeorge syndrome (DGS),¹ velocardiofacial or Shprintzen syndrome (VCFS),² conotruncal anomaly face syndrome (CTAFS),³ Cayler cardiofacial syndrome,⁴ and Opitz G/BBB syndrome.⁵ Deletions of the 22q11.2 region have also been reported in familial and sporadic heart disease, isolated hypoparathyroidism,⁶ psychiatric diseases,⁷ and palatoschisis.⁸ It is unclear at present whether the CATCH 22 phenotype, an acronym for Cardiac defect, Abnormal facies, Thymic hypoplasia, Cleft palate, and Hypoparathyroidism, is the result of a single gene defect or represents a contiguous gene syndrome. It is even possible that variations in the CATCH 22 phenotype are the result of epigenetic factors, differences in genetic background, polymorphisms in the opposing allele, or position effects influencing gene expression over long distances.

Hirschsprung disease (HSCR) or aganglionic megacolon is a congenital disorder characterised by the absence of intrinsic ganglion cells in both the myenteric and the submucosal plexuses of the digestive tract. This aganglionosis results in motility disorders of the colon leading to severe constipation. The prevalence of HSCR is 1 in 5000 live births. The chance of males being affected is four times higher than of females. Microdeletions of

10q11 and 13q33 have been reported in HSCR cases, consistent with the finding of mutations in the RET^{9,10} and EDNRB¹¹ genes, which map within these deletions. In addition, mutations of two other genes have been found associated with the development of HSCR in humans: GDNF, coding for the ligand of the RET protein,^{12,13} and EDN3, the gene coding for endothelin 3, one of the ligands of the endothelin B receptor encoded by EDNRB.^{14,15} It is estimated that mutations in these four genes are responsible for approximately 20% of all HSCR patients (R M W Hofstra, unpublished data). This implies that there must be other genes with mutations predisposing to HSCR.

We report a boy with a deletion of 22q11.2, whose clinical features fit into the CATCH 22 spectrum associated with 22q11.2 deletions, but who also has short segment Hirschsprung disease and paroxysmal hypoventilation. We suggest that the DiGeorge chromosomal region at 22q11.2 may contain a gene that is required for proper development of the enteric nervous system.

Case report

The patient is the second child of a non-consanguineous, healthy 24 year old mother and 27 year old father, both white. At the age of 3 days a rectal suction biopsy was performed in the referring hospital, because of constipation and bile vomiting, but no abnormalities were found. One day later he was referred to the University Hospital. He weighed 2995 g (25th-50th centile), measured 49 cm (25th-50th centile), and had a head circumference of 34.5 cm (10th centile). A cardiac defect was assumed because of tachypnoea and a systolic murmur. A truncus arteriosus type II was diagnosed by ultrasound. Because of this he was screened for deletion of 22q11.2 (see below). Serum calcium was normal and lymphocyte subpopulations had a normal distribution. An antigen stimulation test gave normal results.

Re-evaluation of the rectal biopsy showed an increase of cholinergic fibres. Staining did not allow the presence or absence of ganglionic cells to be judged. A repeated rectal suction biopsy at the age of 7 months, however, showed absence of ganglionic cells and an increase of cholinergic fibres, indicating HSCR. The patient underwent a partial sigmoid resection (a short segment HSCR was found) and a colostomy was performed.

The parents were referred to the Department of Medical Genetics for genetic counselling when the patient was 1 year old. His length was 70 cm (<3rd centile), weight 7.52 kg (10th centile in relation to length), head circumference 47 cm (50th centile), inner canthal

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distance 3.0 cm (97th centile), and outer canthal distance 7.0 cm (25th-50th centile). The skull was brachycephalic and the tip of the nose was broad. The mouth was small with a tented upper lip. The palate was high arched, but without a cleft. The ears showed overfolding of the helices but were otherwise normal (fig 1). Neurological evaluation showed marked hypotonia, especially of the legs. An MRI scan of the brain showed no abnormalities.

At the age of 2 years, he was admitted to hospital because of recurrent periods of one to three minutes of hypoventilation and cyanosis. His pulse was 150/minute and oxygen saturation was 50% on the pulse oximeter during these episodes. He showed mild psychomotor delay. The hypotonia of his legs had decreased.

Re-evaluation of the boy at the age of 4 years showed that his phenotype had evolved towards velocardiofacial syndrome with relatively small eye fissures, a broad nasal tip, open mouth with a tented upper lip, small, round ears with a broad helix, hypernasal speech, regurgitation of food and liquids through the nose, long tapering fingers, and growth retardation. His psychomotor development appeared to be two years behind and he showed some autistic behaviour. Depakine treatment was started for a seizure-like disorder but no epileptic manifestations were seen on EEG.

LABORATORY INVESTIGATIONS

The patient appeared to have a normal 46,XY karyotype. Initial FISH analysis with routine probes for 22q11.2 deletions (M51 and sc4.1) showed a deletion. Additional FISH probes were used to estimate the position of the deletion boundaries. FISH analysis was performed on metaphase spreads using the following probes (in order from proximal to distal):



Figure 1 The patient at the age of 14 months. (Photograph reproduced with permission.)

D22S239, Sc11.1a, TUPLE, M51, M56, Sc4.1, Sc11.1B, COS39, and D22S111-S112. All but D22S239 and D22S111-S112 were found to be heterozygously deleted in the patient. The interstitial deletion spanned from sc11.1A to ZNF74 (COS39), coinciding with the most common type of 22q11.2 deletion.

In view of the presence of HSCR, we decided to screen for mutations in the genes that have been implicated in the aetiology of HSCR so far. High molecular weight DNA was prepared according to standard methods. DGGE analysis of all exons of RET, GDNF, EDNRB, and EDN3 was performed. Mutation analysis of RET,¹⁶ EDNRB,¹⁴ EDN3,¹⁴ and GDNF (Hofstra *et al*, in preparation) was performed as previously described. Apart from already known neutral variants,^{14,16} no mutations were detected in these four genes.

Discussion

In this report we describe a patient with truncus arteriosus, dysmorphic features, and hypotonia in combination with HSCR and paroxysmal hypoventilation and an interstitial deletion of chromosome 22q11.2. The number of symptoms found in patients with the CATCH 22 spectrum is still increasing and, as illustrated by our patient, HSCR might be added to the list of features. Whether these represent true phenotypic associations or merely reflect random events remains to be elucidated. The combination of a 22q11.2 deletion, a CATCH 22 phenotype, HSCR, and paroxysmal hypoventilation has not been reported previously. In a large European collaborative study the combination of a 22q11.2 deletion and HSCR has been found in two patients, one of whom was our patient.¹⁷ We know of one more patient with HSCR who is clinically diagnosed as a VCF patient but whose parents refuse testing for microdeletion 22q11.2 (N Elzenga, Department of Paediatric Cardiology, Groningen, personal communication).

HSCR is not only a heterogeneous, but possibly also a polygenic disease,^{11-13,18} sometimes occurring in association with a wide range of additional anomalies. Interpretation of the paroxysmal hypoventilation as an atypical form of hypoventilation might be in accordance with a number of reports on the combined occurrence of hypoventilation problems (congenital central hypoventilation syndrome (CCSH) or Ondine's curse) with HSCR, a combination referred to as Haddad syndrome.^{19,20}

In the four genes known to be involved in HSCR, we could not detect any mutations in our patient (RET, GDNF, EDNRB, and EDN3). This leaves the possibility that a mutation of another, as yet unidentified gene is responsible for the HSCR in our patient. In HSCR, only about 20% of patients have presumably causative mutations in one of the genes examined. It seems clear that other genes can also lead to the HSCR phenotype or at least contribute to it.

The birth prevalences of HSCR and deletion 22q11.2 are approximately 1 in 5000 and 1 in 4000, respectively. This does not make

coincidence a convincing explanation for the occurrence of three independent cases. Recently, mutations of SOX10 at 22q13 have been found in patients with Shah-Waardenburg syndrome,²¹ a combination of Waardenburg syndrome and Hirschsprung disease (WS4). It is clear, however, that the location of the gene is different from 22q11.2, the site of the deletion in our patient. Moreover, the specific features of Waardenburg syndrome, deafness and pigmentary anomalies, are not part of the phenotype of our patient. However, apart from a 22q11.2 deletion, abnormalities in band q11 of chromosome 22 are known that occur in association with HSCR. A supernumerary chromosome representing an inv dup(22)(q11) causes cat eye syndrome (CES). This syndrome has highly variable clinical features, among them coloboma of the eye, congenital heart defects, and also HSCR.²²⁻²³

Mears *et al*²⁴ reported one CES patient with HSCR (their patient 3) who had three copies of the DiGeorge region. Furthermore, another HSCR patient has been described with partial trisomies 22 and 11 owing to a paternal t(11;22)(q23;q11).²⁵ This patient also showed muscular hypotonia. It is tempting to consider the 22q11.2 deletion responsible for both the CATCH 22 symptoms and HSCR in our patient. How over-representation of a region on the one hand and under-representation of the same region on the other hand may cause HSCR cannot readily be understood. However, a comparable situation to HSCR has been described for the RET gene, where in HSCR patients both activating and inactivating mutations have been found, all suspected of causing the disease.⁹⁻¹⁰⁻²⁶

It is also possible that the 22q11.2 deletion in this patient shows a mutation predisposing to HSCR in the opposite allele. Such a situation has been described in a patient with Bernard Soulier syndrome and velocardiofacial syndrome,²⁷ who had a microdeletion of 22q11.2 together with a mutation in the gene coding for the beta subunit of glycoprotein 1b on the opposite 22q11.2 locus.

The genetics of the CATCH 22 phenotype is complicated. The interstitial deletions are about 2-3 Mb of DNA. In a study of 92 probands in the Hospital for Sick Children in Toronto,²⁸ no relationship could be found between phenotype and deletion size. This is consistent with results from Kurahashi *et al*,²⁹ who described patients with three different types of deletions, who were indistinguishable from each other on clinical grounds. Based on a few patients with unbalanced translocations showing breakpoints in the region, a shortest region of deletion overlap (SRDO) has been defined. The SRDO concept is dwindling, however, owing to reports of patients with a partial phenotype and interstitial deletions that are located far outside the SRDO.³⁰ In addition, the discordance of the phenotype in monozygotic twins with the CATCH 22 deletion underlines the problem with the SRDO concept.³¹ It is still unclear how

haploinsufficiency of a 2-3 Mb region can lead to such a wide range of clinical phenotypes.

The CATCH 22 phenotype is thought to be the result of defective development of neural crest cells originating from the hindbrain or their interaction with the endoderm of the pharyngeal pouches. Several reported genes in this region may be developmental control genes.³²⁻³⁶ Neural crest cells from the same axial level are also crucial for the development of the enteric nervous system. From a developmental point of view, therefore, it could well be that the DiGeorge region in chromosome 22q11.2 contains a gene or a set of genes that are involved in the development of the caudal hindbrain and its crest.

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