A Hirschspring 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.

(Keywords: CATCH 22; Hirschsprung disease; hypoventilation)

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), velocardiofacial or Shprintzen syndrome (VCFS), conotruncal anomaly face syndrome (CTAFS), Cayler cardiovascular syndrome, and Opitz G/BBB syndrome. Deletions of the 22q11.2 region have also been reported in familial and sporadic heart disease, isolated hypoparathyroidism, psychiatric diseases, and palatoschisis. 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 and EDN3 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, and EDN3, the gene coding for endothelin B receptor encoded by EDNRB. 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), 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 patient is 1 year old. His height was 70 cm (<3rd centile), weight 7.52 kg (10th centile in relation to length), head circumference 47 cm (50th centile), inner canthal
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): 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, EDNRB, EDN3, and GDNF (Hofstra et al, in preparation) was performed as previously described. Apart from already known neutral variants, 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. 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, 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.

In the four genes known to be involved in HSCR, we could not detect any mutations in our patient (RET, GDNF, EDN3, 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
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SRDO concept.
deletion underlines the problem with the genes located far outside the SRDO. partial phenotype and interstitial deletions however, owing to reports of patients with a defined. The SRDO concept is dwindling, a few patients with unbalanced translocations from each other on clinical grounds. Based on types of deletions, who were indistinguishable V Waardenburg syndrome,21 a combination of the occurrence of three independent cases. Recently, mutations of SOX10 at 22q13 have been described in a patient with Bernard HSCR in the opposite allele. Such a situation this patient shows a mutation predisposing to this region of deletion overlap (SRDO) has been reported.23 no relationship could be found 27 who had a microdeletion of 22q11.2 deletion in patients with Waardenburg-Hirschsprung disease.19,20 combined Waardenburg type 2 and Hirschsprung phenotype. 5 CES patient with partial HSCR cannot readily be understood. However, the same region on the other hand may cause the 22q11.2 deletion responsible for both the CATCH 22 phenotype and HSCR in our patient.

We would like to thank Pieter van der Vlies, Hendrika Faber, Rein Stulp, and Jan Onsis for technical assistance.