Chromosome 13q deletion with Waardenburg syndrome: further evidence for a gene involved in neural crest function on 13q

G Van Camp, M N Van Thienen, I Handig, B Van Roy, V S Rao, A Milunsky, A P Read, C T Baldwin, L A Farrer, M Bonduelle, L Standaert, F Meire, P J Willems

Abstract

Waardenburg syndrome (WS) is an autosomal dominant disorder characterised by pigmentary abnormalities and sensorineural deafness. It is subcategorised into type 1 (WS1) and type 2 (WS2) on the basis of the presence (WS1) or absence (WS2) of dystopia canthorum. WS1 is always caused by mutations in the PAX3 gene, whereas WS2 is caused by mutations in the microphthalmia (MITF) gene in some but not all families. An association of WS symptoms with Hirschsprung disease (HSCR) has been reported in many families. We report here a patient with characteristics of WS2 and a de novo interstitial deletion of chromosome 13q. We also describe a family with two sibs who have both WS2 and HSCR. In this family, all possible genes for WS and HSCR, but not chromosome 13q, could be excluded. As an association between chromosome 13q and HSCR/WS has been reported previously, these data suggest that there is a gene on chromosome 13q that is responsible for WS or HSCR or both.

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Waardenburg syndrome (WS) is an autosomal dominant disorder, characterised by pigmentary abnormalities of the hair, eyes, and skin. These include a white forelock, premature greying of the hair, heterochromia irides, hypopigmented irises, and white skin patches. Sensorineural hearing impairment is present in 25 to 50% of the patients. With a population frequency of approximately 1/40 000, WS is responsible for about 2% of cases of congenital deafness.¹ WS is subdivided into type 1 (WS1) and type 2 (WS2) on the basis of the presence or absence of dystopia canthorum.²

After the description of a child with WS1 and a de novo inversion of 2q35-q37,³ linkage analysis in several families localised a WS1 gene to this region.⁴⁻⁶ As the Splotch mutation, located in the syntenic region in the mouse, gives rise to white spotting, it has been suggested that WS and Splotch are homologous.⁴ The Splotch phenotype is caused by a mutation in the mouse Pax3 gene, a member of the paired box gene family, known to be involved in embryonic development.⁷ Many mutations have now been found in the homologous human PAX3 gene in different WS1 families.

⁸⁻¹⁰ Pooled data from the Waardenburg consortium indicate that all WS1 families are linked to PAX3.¹¹

One form of WS2 has been localised to chromosome 3p12-p14.1 by linkage analysis.¹² The MITF gene, the human homologue of the mouse microphthalmia (mi) gene, is located in this region.¹³ Mice with mi mutations show reduced pigmentation, microphthalmia, and hearing loss among other abnormalities. Mutations in the MITF gene have recently been found in patients in two WS2 families.¹⁴ However, WS2 is genetically heterogeneous, and some families are not linked to the locus on chromosome 3.

In addition to WS1 and WS2, WS type 3 has been described. WS3, or Klein-Waardenburg syndrome, is an autosomal dominant trait characterised by a WS type 1 phenotype with additional upper limb abnormalities.^{15,16} Linkage analysis has not been performed, but PAX3 mutations have been found in two type 3 families¹⁷ (A P Read, unpublished data), and limb defects are now considered to be a rare pleiotropic manifestation of WS1.¹¹ Many patients have been reported with WS2 symptoms in combination with congenital megacolon (Hirschsprung disease, HSCR).¹⁸⁻²³ The parents of these patients are unaffected and often consanguineous. For this poorly defined, heterogeneous group with apparent recessive inheritance, the term WS type 4 has been proposed.

WS and HSCR are part of a group of diseases called neurocristopathies, which are caused by an abnormal migration of neural crest cells during embryonic development. Apart from the PAX3 and MITF genes, mutations in two proto-oncogenes are known causes of neurocristopathies. Loss of function mutations in the RET proto-oncogene commonly causes HSCR without pigmentary disturbances or deafness, which is inherited as a dominant condition with reduced penetrance.^{24,25} Mutations in the c-KIT proto-oncogene cause piebaldism, characterised by congenital white patches of skin and hair.²⁶

We identified a de novo interstitial deletion of chromosome 13q in a boy with features of WS2, suggesting that a WS gene might be located in the deleted region of chromosome 13q. Although a gene responsible for WS in the deleted region on chromosome 13 could be excluded in several WS2 families, this chromosomal region could not be excluded in a family with WS2 and HSCR.

Department of
Medical Genetics,
University of Antwerp,
Universiteitsplein 1,
B-2610 Antwerp,
Belgium
G Van Camp
M N Van Thienen
I Handig
B Van Roy
P J Willems

Department of
Neurology, Boston
University School of
Medicine, Boston,
USA
V S Rao
L A Farrer

Center for Human
Genetics, Boston
University School of
Medicine, Boston,
USA
A Milunsky
C T Baldwin

University
Department of
Medical Genetics,
St Mary's Hospital,
Manchester, UK
A P Read

Department of
Medical Genetics,
University Hospital of
the Free University of
Brussels, Brussels,
Belgium
M Bonduelle

Institute Spermalie,
Bruges, Belgium
L Standaert

Department of
Ophthalmology,
University of Ghent,
Ghent, Belgium
F Meire

Correspondence to:
Dr Van Camp.

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Figure 1 (A) Idiogram of chromosome 13. The vertical bar indicates the deleted region in the WS2 patient. (B) Partial GTB banded karyotypes of the WS2 patient. The deleted chromosome 13 is shown on the right of each chromosome pair, and the deleted band is indicated on the normal chromosome.

Patients and methods

WS2 PATIENT WITH A CHROMOSOME 13 DELETION

A 6 year old boy presented with unilateral segmental heterochromia of the iris, hypopigmentation of the eye fundus, sensorineural deafness, and delayed psychomotor development (performance IQ was 62 on WPPSI

Table 1 Genotype of the WS2 patient and his parents for chromosome 13 microsatellite markers. Alleles are numbered arbitrarily. Markers are ordered from centromere to telomere, according to the genetic maps of Bowcock et al^{28,31} and the Cooperative Human Linkage Center (CHLC),³⁶ and the physical map of White et al³⁰

Locus	Cytogenetic localisation	Genotype			Conclusion
		Father	Patient	Mother	
D13S126	13q14.1-q14.2	12	12	23	Not deleted
D13S118	13q14.1-q14.2	22	2 or 22	12	Not informative
D13S227*	13q14.3	13	17	37	Not deleted
D13S228*	13q14.3	23	3 or 33	23	Not informative
D13S133*	13q14.3	38	35	35	Not deleted
D13S137*	13q14.3	45	45	55	Not deleted
D13S119	13q14.3-q22	11	1 or 11	11	Not informative
D13S134	13q14.3-q22	35	4	34	Deleted
D13S131	13q14.3-q22	46	4 or 44	14	Not informative
D13S144†	13q14.3-q22	22	1	12	Deleted
D13S317‡		35	4	34	Deleted
D13S121	13q31	22	2 or 22	21	Not informative
D13S140	13q32-q33	12	13	23	Not deleted
D13S125	13q31-q32	21	21	31	Not deleted
D13S122	13q31-q32	13	14	24	Not deleted

* These markers have been localised between D13S118 and D13S119 on a physical map.³⁰
† The exact location of D13S144 relative to D13S134 and D13S131 is not known.^{28,31}
‡ This marker is located 2 cM proximal to D13S121 on the CHLC genetic map.³⁶

scale). A linear skin depigmentation on the legs became evident after Wood lamp examination. Dystopia canthorum was not present (w index of 1.57). There were no signs of HSCR. Clinical examination of the parents showed no signs of WS.

WS2 FAMILIES

All WS2 families used in this study have been described previously.¹¹

FAMILY WITH WS AND HSCR

The male proband of this family developed intestinal obstruction soon after birth. Laparoscopy confirmed the presence of a microcolon, which was shown to be the result of total colonic aganglionosis by microscopic examination. WS was suspected because of cutaneous piebaldism, a white forelock, white streaked eyebrows, white eyelashes, and isochromic pale blue eyes. Dystopia canthorum was absent. Profound bilateral sensorineural deafness was shown by an auditory brainstem response test. Ophthalmological examination showed a normal retina and no other anomalies. Histological examination of the achromatic skin showed a normal epidermis but an absence of melanocytes. Routine cytogenetic analysis did not show any abnormality. The family history included an older brother with similar features of WS who died from long segment HSCR. Neither parent nor three healthy sibs had any sign of WS on further examination. No consanguinity between the parents could be documented, although they both originated from a small Moroccan village of 600 inhabitants.

CYTOGENETIC ANALYSIS

Peripheral blood cultures of the WS2 patient and his parents were synchronised using the methotrexate/bromodeoxyuridine method. High resolution G banding with trypsin/basic fuchsin (GTB) was performed according to Scheres et al.²⁷

MICROSATELLITE ANALYSIS

The following polymorphic dinucleotide repeat chromosome 13q markers were typed: D13S126, D13S118, D13S227, D13S228, D13S133, D13S137, D13S119, D13S134, D13S131, D13S144, D13S317, D13S121, D13S140, D13S125, and D13S122. All these markers have been described before,²⁸⁻³⁰ and are integrated in the genetic^{28,31} or physical³⁰ map of chromosome 13. Experimental procedures for the amplification of microsatellites were as described.³² Microsatellite markers for the PAX3 gene, the RET gene, for markers D3S1261 and D3S1284 flanking the MITF gene, and for the GARB1 marker, located 3 cM proximal of the KIT gene on chromosome 4q, were obtained from the Genome DataBase.³³

LINKAGE ANALYSIS

Linkage analysis was performed as described,¹¹ using the computer programs Liped³⁴ and Link-

Figure 2 Genetic map positions of the chromosome 13q microsatellite markers used in this study, according to Bowcock.³¹ The sex averaged map is shown and distances are indicated in cM.

Table 2 Two point lod scores between WS2 and chromosome 13q markers in WS families from Manchester University (UM) and Boston University (BU) not linked to PAX3

Locus	Family	θ						
		0.0	0.01	0.05	0.10	0.20	0.30	0.40
D13S118	UM2	-4.68	-2.79	-1.30	-0.62	-0.08	0.04	-0.00
	UM26	-7.15	-4.26	-2.27	-1.44	-0.70	-0.33	-0.12
	UM22	-4.11	-1.43	-0.75	-0.47	-0.22	-0.09	-0.03
	UM4	-5.84	-3.35	-1.59	-0.82	-0.17	0.06	0.10
	BU1	-∞	-7.52	-3.49	-1.90	-0.55	-0.02	0.13
	BU3	-∞	-5.32	-2.62	-1.56	-0.64	-0.25	-0.07
	BU11	-∞	-5.02	-2.33	-1.26	-0.37	-0.01	0.09
D13S119	UM2	-11.56	-9.06	-4.71	-2.70	-1.00	-0.36	-0.14
	UM26	-6.58	-2.92	-1.57	-1.01	-0.50	-0.24	-0.10
	UM22	-4.10	-2.79	-1.47	-0.91	-0.41	-0.17	-0.04
	UM4	-2.86	-0.81	-0.16	0.07	0.21	0.22	0.14
	BU1	-∞	-9.52	-4.77	-2.85	-1.15	-0.38	-0.04
	BU3	-∞	-3.55	-1.58	-0.83	-0.25	-0.05	0.00
	BU11	-∞	-3.61	-1.61	-0.82	-0.17	0.07	0.11
D13S144	UM2	-∞	-9.14	-5.62	-3.39	-1.27	-0.36	-0.01
	UM26	-3.05	-1.22	-0.56	-0.30	-0.10	-0.02	-0.00
	UM22	-3.94	-1.14	-0.49	-0.26	-0.08	-0.03	-0.01
	UM4	-0.16	-0.10	0.04	0.12	0.15	0.10	0.03
D13S131	BU1	-∞	-3.91	-1.90	-1.10	-0.42	-0.14	-0.03
	BU3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	BU11	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D13S121	UM2	-7.84	-4.30	-1.93	-0.78	0.12	0.36	0.28
	UM26	-6.20	-2.70	-1.38	-0.86	-0.42	-0.22	-0.09
	UM22	-3.94	-1.14	0.49	-0.26	-0.08	-0.03	-0.01
	UM4	-6.38	-3.87	-2.14	-1.32	-0.58	-0.23	-0.05
	BU1	-∞	-12.11	-6.61	-4.32	-2.16	-1.04	-0.38
	BU3	-∞	-5.17	-2.50	-1.46	-0.60	-0.25	-0.10
	BU11	-∞	-2.51	-1.16	-0.63	-0.18	-0.01	0.04

age.³⁵ Penetrance was set at 93% in both sexes, and a gene frequency of 0.001 was assumed. In the multipoint linkage analysis, intermarker distances were used according to the sex averaged map of Bowcock *et al.*^{28,31}

Results

CYTOGENETIC ANALYSIS

Routine karyotyping of a boy with characteristics of WS2 showed an interstitial deletion of the long arm of chromosome 13. High resolution GTB banding showed that the breakpoints of the deletion were in 13q21.2

and 13q32, respectively (fig 1). No abnormality was found in the parents.

MOLECULAR DELINEATION OF THE DELETION

To correlate the 13q deletion with the genetic map, 15 microsatellite genetic markers spanning the deleted region on chromosome 13q were typed in the WS patient and his parents (table 1). These markers cover approximately 40 cM of the long arm of chromosome 13.³¹ A genetic map containing most of these markers is given in fig 2. The patient is heterozygous for the proximal markers D13S126, D13S227, D13S133, D13S137, and the three most distal markers D13S140, D13S125, and D13S122. Therefore, these loci are not deleted in the patient. No paternal allele of D13S134, D13S144, and D13S317 is present in the patient, indicating that these loci are deleted from the paternal chromosome 13 in the patient (fig 3). The remaining markers D13S118, D13S228, D13S119, D13S131, and D13S121 were not informative. This analysis limits the deleted interval to the region between markers D13S137 and D13S140, a region of less than 30 cM.

LINKAGE ANALYSIS IN WS2

Linkage analysis with chromosome 13q markers was carried out in seven WS2 families that are not linked to the PAX3 gene on chromosome 2.¹¹ Four microsatellite markers spanning the deleted region were analysed (table 1, fig 2). In the University of Manchester (UM) families, D13S118, D13S119, D13S144, and D13S121 were used. In the Boston University (BU) families, D13S131 was used instead of D13S144. The use of different markers should not affect the analysis since both D13S131 and

Figure 3 Autoradiograph illustrating the CA repeat analysis of the WS2 patient (lane 3), his father (lane 1), and his mother (lane 2) for CA repeat markers D13S144 (A) and D13S122 (B). The patient shows a deletion of the paternal allele of D13S144, as he only has the maternal allele 1, whereas his father is homozygous for allele 2. The WS2 patient is heterozygous for D13S122, indicating that this marker is located outside the deletion.

Figure 4 Pedigree structure of the family with WS and HSCR, typed with microsatellite markers for the PAX3 gene, the RET gene, and the GARB1 marker close to the KIT gene (A). Haplotypes are given for two chromosome 3 markers flanking the MITF gene and for six informative chromosome 13 genetic markers from the deleted region in the de novo WS2 patient (B).

D13S144 are unambiguously placed between D13S119 and D13S121.^{28 31}

Table 2 presents two point lod scores between these markers and WS2 in seven different WS2 families. In none of the families tested was there evidence for linkage with chromosome 13q. Multipoint linkage analysis was performed independently in each family (data not shown). Lod scores below -2 were obtained for all inter-marker regions between D1S118 and D1S121 in all three BU families and in family UM 2, excluding the WS gene from this region. In the smaller families UM 26, UM 22, and UM 4 not all inter-marker regions were formally excluded.

WS/HSCR FAMILY

A family with WS and HSCR in two sibs was analysed with microsatellite markers for the PAX3 and RET genes, with marker GARB1, located 3 cM proximal of the KIT gene, and with markers D3S1261 and D3S1284, flanking the MITF gene (fig 4). For all these markers, an identical haplotype was obtained for the WS/HSCR patient and an unaffected sib (fig 4). Under the hypothesis of full penetrance, this excludes all four genes as the cause of WS/HSCR in this family. The family was also typed for a number of chromosome 13q markers, spanning the deletion found in the de novo WS2 patient (fig 4). The patient and his unaffected sibs did not share a parental haplotype for the 13q markers, indicating that the in-

volvement of this chromosomal region as a cause of the disease can not be excluded.

Discussion

In this study we describe a de novo interstitial deletion of the long arm of chromosome 13 in a patient diagnosed with WS2. This suggests that a gene responsible for WS2 is located in this deleted region. Therefore, we performed linkage analysis with chromosome 13 genetic markers spanning the deleted region in seven WS2 families not linked to the PAX3 gene on chromosome 2. However, there was no evidence for linkage with these chromosome 13q markers in either of these WS2 families. Although we could not formally exclude the complete region in the three smaller families, the WS gene was excluded from all inter-marker regions by linkage analysis in the four larger families. These results indicate that the region of chromosome 13q investigated in this study does not contain a locus that is frequently responsible for WS2. After these results were obtained, Tassabehji *et al*¹⁴ reported that WS2 is the result of mutations in the MITF gene in some but not all WS2 families.

Recently, a new locus for HSCR has been mapped to chromosome 13q22 in a single large inbred family. The inheritance pattern in this family is unclear, and both recessive inheritance and dominant inheritance with reduced penetrance are possible.³⁷ The gene causing HSCR in this family is located inside the chromosome

13 region deleted in our patient.³⁷ The authors also incidentally report characteristics similar to WS, including white forelock, bicoloured irides, hearing loss, and hypopigmentation of the skin, in a number of the HSCR patients. Although the authors state that it is likely that these WS characteristics segregate separately from HSCR in this pedigree, the same pedigree has been published independently by others as a combined HSCR/WS kindred resulting from pleiotropic effects of a single mutation.³⁸ This suggested that a gene responsible for both HSCR and WS could be located in this chromosome 13 region.

We therefore analysed a small family with both WS and HSCR with genetic markers in this chromosome 13 region and with possible candidate genes, such as the PAX3, RET, MITF, and KIT genes. Assuming full penetrance, we were able to exclude PAX3, RET, MITF, and KIT mutations as the cause of WS and HSCR in this family. However, analysis with chromosome 13 markers spanning the deleted region in the de novo WS2 patient could not exclude this region. Although the family is too small to derive any linkage information, a chromosome 13q gene could very well be responsible for a combination of WS and HSCR. This is also supported by published data, as chromosome 13 deletions are present in several reports of patients with neural crest dysfunction. Apart from our present case, five cases have been reported of HSCR associated with deletion of the long arm of chromosome 13, with the breakpoints 13q21.2-qter,³⁹ 13q14.1-q22.3,⁴⁰ 13q22.1-q32.1,⁴¹ 13q21.2-q32.3,⁴¹ and 13q32.3-q33.2.⁴² None of these patients was reported to have symptoms of WS, although one did have heterochromia irides.⁴³ Other patients with overlapping 13q deletions have been reported who did not have HSCR.^{41,43} This variability is not incompatible with the presence on 13q of a WS/HSCR gene. It is possible that HSCR in these cases is a recessive entity unmasked by the deletion; in that case patients with a 13q deletion on one chromosome would only express WS/HSCR if they had a second mutation on the non-deleted chromosome. On the other hand, the inheritance could be dominant with reduced penetrance. Both hypotheses would adequately explain why some patients with 13q deletions do not have HSCR. Also, patients with gross rearrangements of 2q35 deleting PAX3 do not necessarily show signs of WS.^{44,45}

In summary, the findings from our study corroborate the published data from which it is clear that (1) WS is associated with HSCR in many cases, (2) HSCR is associated with 13q deletions, (3) a family with characteristics of both HSCR and WS shows linkage to chromosome 13q22. Our study shows that a patient with a 13q21.2-q32 deletion has characteristics of WS, and that WS and HSCR in two sibs of one family might be the result of a mutated gene in the same region of chromosome 13. Taken together, these data strongly suggest the existence of a gene on chromosome 13q that is involved in neural crest cell migration, causing either HSCR, WS, or a combination of both.

The recessive mouse mutation piebald lethal, a mouse model for HSCR, is characterised by striking changes in coat pigmentation and the development of fatal aganglionic megacolon.⁴⁶ The mutation is located on mouse chromosome 14 between the homologue of the human retinoblastoma gene, located on human 13q14.2, and the mouse homologue of the human tyrosine related protein 2, located on human 13q31-32.⁴⁷ If the human-mouse synteny is conserved throughout this region, the human homologue of the gene responsible for mouse piebald lethal is a very strong candidate for the gene involved in neural crest function in this region.

Note added in proof

After this paper was submitted, a mutation was reported⁴⁸ in the endothelin-B receptor (EDNRB) gene in the large inbred HSCR family that is linked to 13q22.^{37,38} Simultaneously, a paper was published⁴⁹ describing mutations in the mouse EDNRB gene producing megacolon and spotted coat colour. These data indicate that the gene on 13q responsible for WS and HSCR is most likely EDNRB.

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