An HDR (hypoparathyroidism, deafness, renal dysplasia) syndrome locus maps distal to the DiGeorge syndrome region on 10p13/14

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Abstract

Partial monosomy 10p is a rare chromosomal condition and a significant proportion of patients show features of DiGeorge syndrome (DGS) and velocardiofacial syndrome (VCFS). A critical haploinsufficiency region for DGS/VCFS was defined on 10p (*DGCR2*). We performed molecular deletion analysis of two further patients with partial monosomy 10p, who showed hypoparathyroidism, deafness, and renal dysplasia or renal insufficiency, but no cardiac defect, cleft palate, or reduced T cell levels. Previously, the combination of hypoparathyroidism, deafness, and renal dysplasia has been proposed to represent a specific syndrome (MIM 146255) under the acronym HDR. In addition to the two patients in this report, at least four published cases with partial monosomy 10p show the triad of HDR and 14 other patients present with at least two of the three features. We therefore conclude that HDR syndrome can be associated with partial monosomy 10p. Based on molecular deletion analysis and the clinical data, we suggest that the DGS/VCFS phenotype associated with 10p deletion can be considered as a contiguous gene syndrome owing to haploinsufficiency of two different regions. Hemizygosity of the proximal region, designated *DGCR2*, can cause cardiac defect and T cell deficiency. Hemizygosity of the distal region, designated *HDR1*, can cause hypoparathyroidism and in addition sensorineuronal deafness and renal dysplasia/insufficiency or a subset of this triad.

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The combination of hypoparathyroidism, deafness, and renal dysplasia was first described in 1992¹ and has been proposed to represent a new syndrome (MIM 146255). Recently, the acronym HDR syndrome has been suggested for this condition, which was found in a patient with partial monosomy 10p.²

Hypoparathyroidism and renal anomalies are also part of the spectrum of DiGeorge syndrome and velocardiofacial syndrome (DGS/VCFS) and are present in a significant proportion of the 49 patients with partial monosomy 10p reported to date.^{2–9} The

molecular deletion analysis of patients with features of DGS/VCFS led to the definition of a critical region on chromosome 10p13/14 (*DGCR2*)^{3–10} in addition to that on chromosome 22q11 (*DGCR1*).

Two patients with a 10p deletion have been reported who showed features of DGS/VCFS but are heterozygous for both *DGCR2* and *DGCR1*.^{5,6} Based on these cases it has been suggested that there are two non-overlapping haploinsufficiency regions on 10p which contribute to the DGS/VCFS phenotype. It is not clear whether the HDR syndrome is part of the DGS/VCFS spectrum or whether it represents a new clinical entity.

Here we present clinical and molecular data of a patient with partial monosomy 10p and HDR syndrome, and the results of molecular analysis of another HDR syndrome patient previously reported.² Our data and review indicate that HDR syndrome is associated with partial monosomy 10p and that a haploinsufficiency region maps distal to *DGCR2*.

Case reports

CASE 1

The female patient (WON) was the first child born to a 23 year old mother and a 28 year old father. There was no family history of recurrent abortions, consanguinity, or mental retardation. She was born at term after an uncomplicated pregnancy with a birth weight of 3080 g (15th centile), a length of 50 cm (20th centile), and a head circumference (OFC) of 36 cm (75th centile). Facial dysmorphism and psychomotor retardation were noted soon after birth.

The following features were noted: downward slanting palpebral fissures, hypertelorism, blepharophimosis, ptosis, epicanthic folds, curled eye lashes, stenosis of the lacrimal ducts, low nasal root, flat nose with anteverted nostrils, double anlage of the left mandibular incisor, high arched palate, micrognathia, small, round, low set, and posteriorly rotated ears with a preauricular sinus, short neck, widely spaced nipples and a right sided accessory nipple, female genitalia with labial synechia, partial syndactyly of toes 2 and 3, clinodactyly of toe 4, and muscular hypotonia (fig 1). CT scan of the brain showed a mildly enlarged ventricular system, but no malformation. Brain stem evoked potentials showed a significant sensorineural bilateral hearing loss affecting all frequencies (–80 dB on the left and –50 dB on the right at 1500 Hz). Hearing loss was more severe at the higher end of the

Figure 1 Patient aged 2 months and 12 years 8 months. (Photographs reproduced with permission.)

frequency range. Laboratory investigations including a metabolic screen were normal. Ultrasound of the kidneys, echocardiography, electrocardiography, electroencephalography, and peripheral nerve conduction velocity were also normal.

The developmental milestones were delayed. She could sit at 15 months and was able to walk at 3 years. From 6 to 8 years she was able to feed herself, to dress, and was toilet trained. At the age of 14 years she did not speak, but understood and followed simple commands. She attended a vocational school.

From the age of 12 years she suffered from several tonic-clonic seizures. When she came to our clinic at the age of 12 years 8 months (fig 1) she was a jittery and hyperactive child with carpopedal spasms. Chvostek and Trousseau signs were positive. Serum calcium was 1.1 mmol/l (normal range 2.1–2.6 mmol/l) with a parathyroid hormone level within the normal range. Owing to the low calcium level without an increased parathyroid hormone level, the diagnosis of idiopathic primary hypoparathyroidism was made. After repeated intravenous infusions of calcium gluconate solution, the therapy was changed to oral administration of calcitriol, magnesium, and calcium, leading to normal calcium levels after two weeks. Immunological studies showed a normal ratio of T and B cells with a reduced proliferative response of T cells to pokeweed mitogen (PWM). Renal insufficiency was noted with a reduced creatinine clearance (22 ml/min, normal range 120–140 ml/min). Nephrosonography showed a diffuse cortical border, but no malformation.

At the age of 12 years 8 months, she had a height of 142 cm (<3rd centile), a weight of 40.4 kg (25th centile), and an OFC of 55.5 cm (90th centile). In addition to the above listed features, she showed hypertelorism (ICD 3.6 cm, >97th centile), synophrys, and thick, bowed eyebrows. She was markedly retarded (level 3–5 years), did not speak, but showed good social adaptation.

Chromosome analysis of peripheral lymphocytes using standard procedures showed the karyotype 46,XX,del(10)(p13). Parental karyotypes were normal. A microdeletion of 22q11 was excluded by FISH using a cosmid probe corresponding to D22S75, which maps within the common *DGS1* deletion region.

CASE 2

The clinical features of the female patient (WAB) at the age of 2 years have been described previously.² She presented with ventricular septal defect, hypoparathyroidism, sensorineural deafness, and renal dysplasia.

When recently seen by us at 7 years 9 months of age her weight, height, and OFC were 17.0 kg (3rd–10th centile), 109.0 cm (below the 3rd centile), and 47.5 cm (below the 3rd centile), respectively. Developmental milestones were delayed. She was able to walk at 3 years 1 month. Between 6 and 7 years 6 months, she was toilet trained, able to dress herself, and to play simple games. At present she is attending a special school for deaf children. On examination, she had hypertelorism, low set ears, a low nasal bridge, bilateral clinodactyly of the 4th toes, and mild hypertrichosis. Serum calcium and phosphate were 9.7 mg/dl (normal range 8.8–10.3 mg/dl) and 5.0 mg/dl (normal range 3.8–6.5 mg/dl), respectively with 1 α hydroxy vitamin D treatment (1.0 μ g/day). Her auditory threshold was 60–80 dB by conditioned oriented reflex tests. Serum urea nitrogen and creatinine were 15.0 mg/dl (normal range 5.0–18.2 mg/dl) and 0.4 mg/dl (normal range 0.2–0.5 mg/dl). No imaging studies of the urinary system were done after the first evaluation.² A microdeletion of 22q11 was excluded by FISH using a cosmid probe corresponding to D22S75.

Molecular analysis

METHODS

PCR analysis of six polymorphic (CA)_n repeats (GATA-P19252, GATA-P34271, AFM154y64, D10S1720, D10S1216, D10S223) was performed in order to determine the extent and the parental origin of the de novo deletion in patient WAB. FISH analysis using YAC and PAC clones was performed as described elsewhere.³ The PACs were isolated from the library of P DeJong (Sanger Centre, Hinxton).

RESULTS

FISH analysis in WON showed hemizygosity for YAC 809F9 (D10S552) mapping to 10p15 (parental DNA was not available). She was hemizygous for PACs 414O17 (WI-600) and 123I2 (D10S1720), but dizygous for PACs 837N7, 107I17 (D10S547), 106G1, 204F19 (WI2389), and 323N1 (D10S1705) (fig 2A). Thus, the breakpoint of the terminal deletion of WON maps between D10S1720 (deleted) and WI2389 (not deleted).

The distal boundary of *DGCR2* has been defined by the distal breakpoint interval WI600-WI2389 of the interstitial deletion of patient MEG (P3, CH95-199).^{3,5,10} In order to characterise the boundary of *DGCR2* further, we performed FISH analysis in MEG with additional PACs and defined his breakpoint to

Figure 2 Molecular characterisation of 10p deletions in 10 patients with features of DGS/VCFS syndrome and HDR syndrome. (A) The results of fine mapping of breakpoint intervals by FISH with seven PACs show that the deletion of MEG does not overlap with the deletion of WON and that the breakpoint of WON maps at least 200–300 kb distal from DGCR2. Deleted PACs are indicated as open boxes, non-deleted PACs as closed boxes. (B) The patients indicated with a three letter acronym were studied by us. Data from patients MEG, AMS, and MAR have been reported previously.³ Clinical data of WAB have been reported previously² and WON is a new patient. The molecular and clinical data from the other five patients were taken from published reports. Patient CH92-092 was reported by Gottlieb et al.,⁵ DW was reported by Dasouki et al.,⁶ and C1 and C2 are cases 1 and 2 described by Van Esch et al.¹¹ The features of the patients are given according to the acronyms of the DGS/VCFS spectrum (CATCH=Cardiac defect, Anomaly of face, Thymic hypoplasia, Cleft palate, Hypocalcaemia/Hypoparathyroidism) and HDR syndrome (Hypoparathyroidism, Deafness, Renal anomaly). Distances of markers were taken from the LDB map (<http://cedar.genetics.soton.ac.uk/pub/chrom10/map.html>). The regions for DGS (DGCR2) and HDR syndrome (HDR1) are indicated. Solid horizontal bars indicate regions not deleted in the patients, breakpoint intervals are drawn as hatched bars, and deleted regions as open bars.

map between PAC106G1 (not deleted) and 204F19 (deleted). This showed that both WON and MEG were dizygous for PACs 837N7, 107I17, and 106G1 and that the terminal deletion of WON does not overlap with the interstitial deletion of MEG. The exact distance between the two deletions is not known, but should be at least 200–300 kb in size.

Patient WAB showed lack of the paternal allele for GATA-P19252, GATA-P34271, and D10S1720 (not shown). He was heterozygous for D10S223. FISH analysis using PACs 204F19 (WI-2389), 414O17 (WI-600), and 123I2 (D10S1720) showed signals on the normal but not on the deleted chromosome 10 for each PAC, while for PAC 323N1 (D10S585) signals on both the normal and the deleted chromosome 10 were detected (fig 2). Thus, the terminal deletion of patient WAB overlaps DGCR2 with a breakpoint between WI-2389 (deleted) and D10S585 (present) giving a deletion size of about 30 cM.

Discussion

HDR SYNDROME IS ASSOCIATED WITH PARTIAL MONOSOMY 10p

Patient WON showed the phenotypic characteristics of partial monosomy 10p including developmental delay, epicanthic folds, downward slanting palpebral fissures, hypertelorism, ptosis, flat nasal bridge, micrognathia, dysmorphic, low set ears, and hand/foot abnormalities. She also presented with hypocalcaemia/hypoparathyroidism, which is a typical feature of the DGS/VCFS spectrum. However, she did not show other typical features of DGS/VCFS, such as a cardiac defect, cleft palate, and abnormal T cell levels, although a reduced response to PWM was observed. In addition to hypoparathyroidism, she presented with sensorineural deafness and renal insufficiency. Autosomal dominant familial hypoparathyroidism, sensorineural deafness, and renal dysplasia with a variable expression pattern in at least six affected patients was described by

Bileous *et al.*¹ It has been proposed that this condition represents a distinct clinical entity (MIM 146255), and the acronym HDR syndrome was suggested.² Two subjects of the family of Bileous *et al.*¹ showed cystic kidneys with normal glomerular filtration rate, and three subjects (one of them has normal hearing) showed bilateral renal dysplasia with a reduced filtration rate and abnormal serum creatinine concentrations. The morphological renal abnormalities found in this family were not observed in patient WON, who showed a diffuse cortical border as the only ultrasonographic abnormality (a biopsy was not performed). However, she showed a reduced glomerular filtration rate and abnormal serum creatinine levels on repeated investigations. Because she showed this renal anomaly in combination with hypoparathyroidism and deafness, we suggest that she can be defined as having HDR syndrome (MIM 146255).

The second patient in this report (WAB) also presented with HDR syndrome.² In addition, four published cases with partial monosomy 10p also presented with the triad characterising HDR syndrome.^{3,4,9,12} As illustrated in table 1, the renal abnormalities are variable and can be asymmetrical. They involve dysplastic kidney, cystic kidney, aplastic kidney, and hydronephrosis. The deafness is of the sensorineural type in most cases, but conductive hearing loss was described in at least one case.

In addition to the six patients known to present the HDR triad, at least 14 patients with a 10p deletion have been described with two of the HDR features (HD n=4, HR n=8, DR n=2). These findings suggest that there is a haploinsufficiency region for HDR syndrome on 10p.

HDR SYNDROME MAPS DISTAL TO *DGCR2*

The breakpoint of the terminal deletion of patient WON presenting with the triad of HDR syndrome was mapped at least 200–300 kb distal to *DGCR2*, the distal boundary of which is defined by the distal breakpoint of the interstitial deletion of patient MEG (fig 2A). Thus, the phenotype in WON might be caused by

haploinsufficiency of a gene(s) distal to *DGCR2*, although position effects cannot be excluded.

Further support for an HDR locus distal to *DGCR2* is given by a patient (C2, fig 2B) who presented with all three features of HDR syndrome¹² and a terminal deletion distal to *DGCR2*.¹² Another patient with HDR syndrome (C1, fig 2B) has a cytogenetically balanced insertional translocation 10p;8q with breakpoints distal and proximal to *DGCR2*, leaving *DGCR2* intact.¹¹ The distance between the 10p breakpoint of the latter patient and *DGCR2* has not been determined in detail, but can be estimated to be at least 1–2 Mb (HVE, PL, unpublished data). This makes a position effect unlikely. In addition, two patients presenting with two out of the HDR syndrome features (patient DW with HD, patient CH92-092 with HR) have been reported to be dizygous for *DGCR2*.

TWO DIFFERENT 10p REGIONS CONTRIBUTE TO THE DGS2 PHENOTYPE

Phenotypic differences between the DGS/VCFS phenotypes associated with monosomy 22q11 (DGS1) and monosomy 10p (DGS2) involve a higher frequency of renal abnormality and deafness and more heterogeneous cardiac defects in DGS2.^{3,11} Moreover, the type of deafness is different in both groups. While about one third of the patients with DGS1 have a conductive hearing loss owing to recurrent otitis media or palatal abnormalities or both, the deafness in monosomy 10p is of the sensorineural type. This raised the question of different molecular and pathogenetic mechanisms underlying DGS1 and DGS2. Genotype-phenotype analysis of the patients from this report and from the published cases suggest that the features of DGS2 are caused by haploinsufficiency for two different regions (fig 2).

Patients MEG and MAR have an interstitial deletion, they are hemizygous for *DGCR2*, and show a cardiac defect,³ as do a significant proportion of patients with a terminal deletion involving *DGCR2*. Patient WAB is partially deleted for *DGCR2* and showed ventricular septal defect (VSD). In contrast, none of the

Table 1 Features of the DGS/VCFS and the HDR syndrome spectrum of nine patients with structural abnormality of 10p

	Patient								
	WAB	WON	C2	DW	CH92-092	C1	MEG	AMS	MAR
Reference	2, this report	This report	11, 12	6	5	11	14	3	13
Sex	M	F	F	M	F	M	F	F	M
10p aberration	del(10) (p13)	del(10) (p13)	del(10) (p13)	del(10) (p13)	del(10) (p13)	ins(8;10) (p13)	del(10) (p12p13)	del(10) (p12p13)	del(10) (p11p13)
Cardiac defect*	VSD	—	—	—	—	ins(10;8) (p13)	VSD, ASD	—	ASD
Anomaly of face	+	+	+	+	+	+	+	+	+
T cell abnormality	—	—	—	+	+	—	+	—	+
Cleft palate	—	—	—	—	—	—	+	—	—
Hypocalcaemia/hypoparathyroidism	+	+	+	+	+	+	—	—	+
Deafness†	s	s (r -85 dB, l -50 dB)	s (-70 dB)	c (severe)	?	s (-85 dB)	—	—	+
Renal defect‡	DK(r)	RI	AK(r)	—	DK(r)	AK(l)	—	—	HN
DGS/VCFS spectrum	CAH	AH	AH	ATH	ATH	A	CATC	A	CATH
HDR spectrum	HDR	HDR	HDR	HD	HR	HDR	—	—	HDR
Molecular findings§	<i>DGCR2</i> partially deleted	<i>DGCR2</i> present	<i>DGCR2</i> present	<i>DGCR2</i> present	<i>DGCR2</i> present	<i>DGCR2</i> present	<i>DGCR2</i> deleted	<i>DGSCR2</i> deleted	<i>DGSCR2</i> deleted

*ASD = atrial septal defect, TOF = tetralogy of Fallot, VSD = ventricular septal defect.

†If known, the type of deafness is given: c = conductive, s = sensorineural.

‡AK = aplastic kidney, DK = dysplastic kidney, HN = hydronephrosis, RI = renal insufficiency, VR = vesicoureteral reflux, l = left side, r = right side.

§The molecular data are presented in fig 2.

patients with a more distal deletion not involving *DGCR2* has a cardiac defect. Therefore, cardiac defects can be associated with hemizygosity for *DGCR2*.

A T cell deficiency has been described both in patients who are hemizygous for *DGCR2* (MEG, MAR) and in patients who are dizygous for *DGCR2* but hemizygous for a more distal segment (DW, CH92-092), suggesting that there are two haploinsufficiency regions for T cell defect. Hypoparathyroidism/hypocalcaemia is the most common feature (in some patients with hypocalcaemia no parathyroid hormone level was given) and was found in four deletion patients, who are dizygous for *DGCR2*, but hemizygous for a more distal region (WON, C2, DW, CH92-092) (fig 2B). In contrast, patients MEG and AMS, who are hemizygous for *DGCR2* but dizygous for the region distal to *DGCR2*, did not show hypoparathyroidism. This provides evidence for a locus for hypoparathyroidism mapping distal to *DGCR2*. The features sensorineural deafness (present in WON, C2, DW, and MAR) and renal anomaly (present in WON, C2, CH92-092, and MAR) can also be associated with a deletion which maps distal to *DGCR2*.

The smallest region of deletion overlap associated with the triad of hypoparathyroidism, deafness, and renal anomaly (HDR) can be defined by the deletions of patients WON and C2 (terminal deletion) and MAR (interstitial deletion) (fig 2). This rather large interval between WI2389 (WON, C2) and D10S552 (MAR) can be further narrowed by the breakpoints of patients CH92-092 and C1. Considering patient CH92-092, who has hypoparathyroidism and renal anomaly, as affected with partial HDR syndrome, the HDR syndrome deletion region can be further narrowed to map distal to D10S226. Interestingly, the distal 10p breakpoint of patient C1, who carries a complex rearrangement associated with HDR syndrome maps between D10S189 and WI-2389.¹¹ This suggests that the HDR syndrome of this patient is caused by haploinsufficiency for one or more gene(s) located in this breakpoint region, which would narrow the distal boundary of the HDR syndrome deletion region to lie proximal to D15S189. Cloning of the distal 10p breakpoint of C1, which could involve an as yet undetectable deletion, might indicate candidate genes for HDR syndrome.

Because the HDR syndrome deletion region between D10S189 and D10S226 includes a

gap within the Whitehead YAC contig, the map size can only provisionally be estimated to be 2-4 Mb.

Based on the growing number of patients with 10p deletions and features of the DGS/VCFS spectrum and on the data presented in this study, we suggest that DGS2/VCFS2 can be considered as a contiguous gene syndrome resulting from haploinsufficiency of two different regions. Hemizygosity of the proximal region, designated *DGCR2*, can cause cardiac defect and T cell deficiency. Hemizygosity of the distal region, designated *HDR1*, can cause hypoparathyroidism and in addition sensorineural deafness and renal dysplasia/anomaly or a subset of this triad. This hypothesis might be useful in elucidating the molecular defects in DGS2/VCFS2.

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- 1 Bileous RW, Murty G, Thakker RV, *et al.* Autosomal dominant familial hypoparathyroidism, sensorineural deafness, and renal dysplasia. *N Engl J Med* 1992;357:1069-74.
- 2 Hasegawa T, Hasegawa Y, Aso T, *et al.* HDR syndrome (hypoparathyroidism, sensorineural deafness, renal dysplasia associated with del(10)(p13)). *Am J Med Genet* 1997;73:416-18.
- 3 Schuffenhauer S, Lichtner P, Peykar-Derakhshandeh P, *et al.* Deletion mapping on chromosome 10p and definition of a critical region for the second DiGeorge syndrome locus (DGS2). *Eur J Hum Genet* 1998;6:213-25.
- 4 Schuffenhauer S, Seidel H, Oechsler H, *et al.* DiGeorge syndrome and partial monosomy 10p: case report and review. *Ann Genet* 1995;38:162-7.
- 5 Gottlieb S, Driscoll DA, Sellinger B, Emanuel B, Budarf ML. Characterization of 10p deletions suggests two non-overlapping regions contribute to the DiGeorge syndrome phenotype. *Am J Hum Genet* 1998;62:495-8.
- 6 Dasouki M, Jurecic V, Phillips JA, Whitlock JA, Baldini A. DiGeorge anomaly and chromosome 10p deletions: one or two loci? *Am J Med Genet* 1997;73:72-5.
- 7 Kato Z, Kato T, Kondo N, Orii T. Interstitial deletion of the short arm of chromosome 10: report of a case and review of the literature. *Jpn J Hum Genet* 1996;41:333-8.
- 8 Sunada F, Rash F, Tam DA. MRI findings in a patient with partial monosomy 10p. *J Med Genet* 1998;35:159-61.
- 9 Hon E, Chapmann C, Gunn T. Family with partial monosomy 10p and trisomy 10p. *Am J Med Genet* 1995;56:136-40.
- 10 Daw SCM, Taylor C, Kraman M, *et al.* A common region of 10p deleted in DiGeorge and velocardiofacial syndromes. *Nat Genet* 1996;13:458-60.
- 11 Van Esch H, Groenen P, Daw S, *et al.* Partial DiGeorge syndrome in two patients with a 10p rearrangement. *Clin Genet* 1999;55:269-76.
- 12 Fryns J-P, de Muelenaere A, Van den Berghe H. Distal 10p deletion syndrome. *Ann Genet* 1981;24:189-90.
- 13 Obregon MG, Mingarelli R, Giannotti A, Di Comite A, Spedicato FS, Dallapiccola B. Partial deletion 10p syndrome. *Ann Genet* 1992;35:101-4.
- 14 Lipson A, Fagan K, Colley A, *et al.* Velo-cardio-facial and partial DiGeorge phenotype in a child with interstitial deletion at 10p13 - implications for cytogenetic and molecular biology. *Am J Med Genet* 1996;65:304-8.