Precise localisation of 3p25 breakpoints in four patients with the 3p- syndrome

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Abstract
In patients with the 3p- syndrome, hemizygous deletion of 3p25-pter is associated with profound growth failure, characteristic facial features, and mental retardation. We performed a molecular genetic analysis of 3p25 breakpoints in four patients with the 3p- syndrome, and a fifth patient with a more complex abnormality, 46,XY,der(3)t(3;?)(p25.3;?). EBV transformed lymphoblasts from each of the patients were initially characterised using fluorescent in situ hybridisation (FISH) and polymorphic microsatellite analyses. The 3p- chromosome from each patient was isolated from the normal chromosome 3 in somatic cell hybrid lines and subsequently analysed with polymorphic and monomorphic PCR amplifiable markers from 3p25. The analysis clearly shows that all five breakpoints are distinct. Furthermore, we have identified yeast artificial chromosomes that cross the 3p25 breakpoints of all four 3p-patients. Two of the patients were deleted for the von Hippel-Lindau (VHL) tumour suppressor gene, although neither has yet developed evidence of VHL disease. The patient with the most centromeric breakpoint, between D3S1585 and D3S1263, had the most severe clinical phenotype including an endocardial cushion defect that was not observed in any of the four patients who had more telomeric breakpoints. This study should provide useful insights into critical regions within 3p25 that are involved in normal human growth and development.

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Key words: 3p- syndrome; 3p25 breakpoints.

The 3p- syndrome comprises people who have a recognisable pattern of dysmorphic features with growth and mental retardation caused by a distal deletion of the short arm of chromosome 3. A total of 23 cases have been reported and the characteristic features include low birth weight, trigonocephaly, hypotonia, psychomotor and growth delay, blepharoptosis, telecanthus, downward slanting palpebral fissures, micrognathia, and mental retardation.[1-10] More variable features include postaxial polydactyly, renal anomalies, cleft palate, congenital heart defects, preauricular pits, gastrointestinal anomalies, and sacral dimples. The von Hippel-Lindau disease tumour suppressor gene is localised within 3p25.3 and patients hemizygous for this gene would be expected to develop VHL related tumours, including retinal and cerebellar haemangioblastomas and renal cell carcinoma.[11]

The initial reports of patients with the 3p-syndrome delineated the clinical manifestations of this congenital disease and described the cytogenetic characterisation of the respective 3p25 breakpoints. In virtually all cases the breakpoints appeared, by cytogenetic analysis, to be identical. However, Mowrey et al[5] and Phipps et al[9] used polymorphic markers from 3p25-pter for the analysis of transformed lymphoblastoid cell lines to localise molecularly the breakpoints in several 3p- patients. The studies showed that the extent of the 3p- deletion was variable among patients. Moreover, Phipps et al[9] obtained evidence to suggest that a gene involved in normal cardiac development may lie in the interval between D3S1250 and D3S18.

In this report we describe the molecular genetic characterisation of the 3p25 breakpoints in four patients with the 3p- syndrome. We also characterised a fifth patient who is apparently hemizygous for 3p26-pter sequences by virtue of an unbalanced chromosome translocation, 46,XY,der(3)t(3;?)(p25.3;?). Transformed lymphoblastoid lines were developed from all five patients and we then constructed interspecific somatic cell hybrid lines to isolate each of the der(3) chromosomes from the the normal human chromosome 3. The isolated 3p- chromosomes were characterised using polymorphic and monomorphic PCR markers from 3p25. Since this region is relatively rich in probes, we were able to localise and distinguish all five breakpoints precisely. This work should aid in the localisation of genes from this chromosomal region that play a role in normal human growth and development.

Material and methods

PATIENT ASCERTAINMENT
Two previously unreported patients (TL and MN) were ascertained through the Thompson-Okanagan Genetic Outreach Program from the Department of Medical Genetics at the University of British Columbia. Twenty to 45 ml of peripheral blood was obtained by venepuncture from patients and parents in both families. DNA was purified

from 10-30 cc of blood[12] while the remainder of the blood provided lymphocytes for immortalisation with Epstein-Barr virus (EBV).

Three previously described 3p- patients were also analysed. Two of the patients (LD and DZ) were originally described by Ramer *et al.*[6] One of the patients, DR, was previously described by Tazelaar *et al.*[7]

GENERATION OF TRANSFORMED
LYMPHOBLASTOID CELL LINES

Viable lymphocytes from the five patients were transformed with EBV into continuous lymphoblastoid cell lines using the method described by Neitzel.[13]

FLUORESCENCE IN SITU HYBRIDISATION ANALYSIS

Microscope slides with interphase cells and metaphase chromosome spreads were prepared from cultures of the established lymphoblast cell lines. Actively growing cells were arrested in metaphase with a 90 minute incubation in 10 µg/ml colcemid and 10 µg/ml ethidium bromide.

The two probes used for the initial FISH analysis were cosmid cA479, previously shown to span the D3S18 locus in 3p25.3,[14] and cosmid cA243 which has been localised to 3p24.2.[15] We also used a chromosome 3 specific subtelomeric probe (Oncor) to determine if the der(3) chromosomes present in the two new patients contained subtelomeric sequences indicative of an interstitial deletion of 3p25-p26 sequences.

Biotin labelled probes were detected using a fluorescein isothiocyanate (FITC)-avidin conjugate and the chromosomes were stained with propidium iodide according to the instructions contained in the ONCOR, Inc, Biotin Detection Kit. Fluorescent signals were analysed using a Zeiss axioscope coupled with a Photometrics CCD camera. The images were captured and merged using the Gene-Join graphics software package (Yale University) on a MacIntosh Quadra computer system.

ISOLATION OF DER(3) CHROMOSOMES IN SOMATIC CELL HYBRIDS

The human lymphoblastoid or fibroblast donor cells were fused with the mutant Urd-C chinese hamster ovary cell line that is deficient in orotate phosphoribosyl transferase and OMP decarboxylase activities, which has been localised to the long arm of chromosome 3.[16] After fusion, the cells were grown in Hams' F12 medium with 10% dialysed serum that lacks endogenous uridine. Oubain (3 × 10^{-7} mol/l) was added to select against the parental human cells. Four to six weeks after fusion, individual colonies were isolated and subsequently expanded for DNA extraction.

PCR ANALYSIS OF ISOLATED DER(3)
CHROMOSOMES

Hybrids containing only the der(3) chromosomes were analysed with the following PCR amplifiable markers from 3p: D3S659 (3p13), D3S1289 (3p21), D3S1259 (3p25), D3S1252 (3p25), D3S1255 (3p25), D3S1110 (3p25), D3S1585 (3p25), D3S1263 (3p25), D3S587

(3p25), D3S1038 (3p25), ATP2B2/D3S601 (3p25), D3S1317 (3p25), STS amplicons for the 3' and 5' ends of the VHL gene (3p25.3), an STS derived from CEPH YAC 875D2, D3S1597 (3p25), D3S18 (3p25), D3S1304 (3p25), and D3S1297 (3p26). All of these markers, except the 875D2 STS, were previously ordered relative to each other and corresponding YAC clones were identified for many of them.[17]

Oligonucleotide primers for the highly polymorphic loci were purchased from Research Genetics, Inc, and amplification conditions were as described by Research Genetics. The ATP2B2 STS primers (GDB ID G00-251-789) were synthesised according to the report of Naylor *et al.*[18] The 875D2 STS primers produce a 223 bp CA containing amplicon with low heterozygosity and were synthesised according to data from the Whitehead Institute/MIT Center for Genome Research.[19] The VHL 5' and 3' primer pairs were synthesised for exon 1 and exon 3 amplicons, respectively, from the VHL disease gene sequences reported by Latif *et al.*[11] VHL 5' (exon 1) primers: A1 5'- ATA GTG GAA ATA CAG CAA CGA GTT GGC CTA GCC TCG C -3'. B1 5'- CGG CCT CGT CCC AGT TCT C -3'. VHL 3' (exon 3) primers: A5 5'- TTC CCT GTA CTG AGA CCC TAG T -3'. B5 5'- TAC CAT CAA AAG CTG AGA TGA AAC AGT GTA AGT -3'.

Results

CLINICAL FINDINGS

Patient 1 (TL), a girl, was born weighing 2523 g to healthy parents after a pregnancy marked by polyhydramnios and intrauterine growth retardation. At 9 months of age, her height, weight, and head circumference were all below the 5th centile. Examinations at ages 9 months, 2, 5, and 6 years showed dysmorphic features, short stature, developmental delay with poor speech, hypotonia, and seizure disorder. The patient was noted to have hirsute skin, pectus excavatum, puffy dorsum of the feet and hands, slight clinodactyly, short fingers, low hairline in front, midface hypoplasia, low set ears with hearing problems, high arched palate, widely spaced eyes, marked bilateral ptosis, epicanthic folds with upward slanting palpebral fissures, a relatively long philtrum, and a significant overbite with a tented upper lip. At 6½ years, she showed developmental delay in all areas having gross motor skills at the 3-4 year old level and speech skills at the 2-3 year old level. This patient had no congenital heart problems, nor did she show any other organ abnormalities. Chromosomal analysis showed 46,XX,del(3)(p25→pter).

Patient 2 (MN), a boy, was delivered weighing 2466 g to healthy parents who were 29 (mother) and 23 (father) years old. At birth the infant was shocked and required resuscitation. He had low Apgar scores with a slow response to ventilation and the placenta was noted to be small and abnormal in appearance. The infant at 4 months had a poor sucking reflex and was at the 10th-15th centile for height, weight, and head circumference. Examination showed a

Table 1   Clinical phenotypic features of the four 3p-syndrome patients in this study. Clinical data on the fifth patient, MN, who has a der(3)(t(3;?), are not included. No entry indicates that trait was not specifically addressed in the patient's clinical report. Superscript M seen for patient DR indicates the phenotype of the 3p- mother is discordant with the phenotype of the 3p- son for that trait

	DR	TL	LD	DZ
Developmental delay	+ +	+	+	+ +
Low set/abnormal ears	+	+	+	+
Growth retardation	+ / –	+	+	+
Hypotonia	–	+	+	+
Apparent microcephaly	–	+	+	+
Micrognathia	–	+	+	+
Downturned/thin upper lip	–	+	+	+
High arched palate	–	+		+
Hypertelorism/telecanthus	+	+	+	–
Hearing impairment	+	+	+	
Long philtrum	+ M	+	+	+
Ptosis	– M	+	+	–
Abnormal nose	+ M	–	+	+
Seizures	+ M	+	–	–
Clinodactyly	–	+	+	–
Rocker bottom feet	–	–	+	–
Renal abnormalities	–	–	+	–
Preauricular pits	+	–	–	–
Colon rotated			+	
Cardiovascular defect	–	–	–	+

right palmar crease, micrognathia, bilateral equinovarus, gross bilateral inguinal hernias, slightly distended abdomen, and congenital laryngeal stridor (laryngomalacia).

At 9 and 18 months of age, patient MN had a head circumference in the normal range. At 9 months of age, he had increased muscle tone with myoclonic jerks. He was noted to have developmental delay in all areas. In addition, the patient had a double hair whorl, high arched palate, and fused right incisor teeth. Unusual facial features included a high forehead with bitemporal narrowing, microphthalmia, small palpebral fissures, closely set eyes, tight skin over the face with medial flaring of the brows, and a broad nasal bridge with a tiny nasal tip and anteversion of the nostrils. The limbs were abnormally tapered to the small hands and feet with marked tapering of fingers, adducted thumbs, single creases on the fifth

fingers, hyperconvex fingernails, and a short, broad second finger. The legs were externally rotated with rigid extension of the knee. No cardiac murmur was detected and his hearing appeared unaffected. Cytogenetic analysis showed an unbalanced rearrangement at the distal end of the short arm of chromosome 3: 46,XY,der(3)t(3;?)(p25.23;?). This patient is therefore not a true 3p- patient and many of his clinical symptoms may be because of duplication of sequences present on the unassigned chromosome translocated onto the chromosome 3 with a terminal deletion.

Patient 3 (DR) was previously described by Tazelaar *et al.*[7] This patient, a boy, was born to a mother who also had the 3p- syndrome. Like his mother, he had no congenital heart problems.

Patient 4 (LD), a girl, was previously described by Ramer *et al.*[6] This patient also had no congenital heart problems.

Patient 5 (DZ), a boy, was also previously described by Ramer *et al.*[6] This patient was the most severely affected of the five patients, and also had a complete endocardial cushion defect.

Table 1 summarises the clinical findings of the four true 3p- patients.

FISH CHARACTERISATION OF LYMPHOBLASTOID CELL LINES

Cell lines from all five patients were analysed with cosmids cA243 and cA479. All five cell lines generated two signals with the 3p24.2 cA243 cosmid probe (data not shown). Four of the cell lines produced only one signal per diploid cell with the 3p25.3 probe cA479, and the cell line from patient MN generated two signals with the cA479 probe (data not shown). Thus, the breakpoint in patient MN was distal to cA479, whereas the breakpoints in the four true 3p- patients were distal to cA243, but proximal to cA479. Cell lines from DR, TL, and MN were also analysed with the chromosome 3 specific subtelomeric probe from ONCOR, Inc. In all three cell lines, only one signal per diploid cell was generated with this probe (data not shown) indicating that these der(3) chromosomes are not the result of interstitial deletions of 3p25-p26 sequences, but probably represent terminal deletions (however, the possibility of cryptic chromosome translocations was not ruled out with this analysis).

MOLECULAR CHARACTERISATION OF LYMPHOBLASTOID CELL LINES

Transformed lymphoblastoid cell lines from all five 3p- patients, and many of their parents, were partially characterised by allelotype analysis using PCR amplifiable polymorphic microsatellite markers from the 3p25-pter region of chromosome 3. An analysis of the transformed lymphoblastoid cell lines obtained from the two new patients, MN and TL, indicated that their der(3) chromosomes were both paternally derived. DNA was not available from the parents of DZ. We suspected maternal inheritance of DR's 3p- chromosome because his mother also has the 3p- syndrome

Table 2   Allelic PCR analysis using polymorphic markers from 3p. The allelotype analysis of somatic cell hybrids containing the der(3) from each of the five patients indicates the successful isolation of the 3p- chromosome in each case. Also included in this table are the allelotype analysis on the original transformed lymphoblastoid cell lines, as well as the parents (when available) for each patient. The analysis shows the parental origin of the 3p-chromosome in the four patients for whom DNA from the parents was available. Hybrid clones UMN-25, UDR-2, UTL-6, ULD-7, and UDZ-22 contain the isolated der(3) chromosomes from patients MN, DR, TL, LD, and DZ, respectively. Hybrids containing the isolated normal chromosome 3 from each of the patients were also analysed (data not shown)

3p telomere←								→centromere
Locus D3S-	1297	1304	1597	1317	1038	1263	1289	659
MN	4 -	4 4	3 4	4 5	1 2	3 5	1 6	2 5
M-MN	3 4	3 4	1 2	3 5	1 2	3 6	2 6	1 2
F-MN	1 3	3 4	4 4	3 4	1 2	5 5	1 5	2 5
UMN-25 pat	-	ND	4	4	1	5	1	5
DR	3 -	4 -	3 -	1 2	1 3	5 6	3 2	6 1
M-DR	3 -	5 -	6 -	1 2	3 3	6 7	4 2	8 6
UDR-2 mat	-	-	-	2	3	6	2	6
TL	3 -	2 -	4 -	2 2	2 6	3 4	5 5	4 4
M-TL	3 3	1 2	3 4	2 3	2 5	3 5	1 5	2 4
F-TL	1 2	2 4	1 4	2 2	4 6	4 6	1 5	4 4
UTL-6 pat	-	-	-	2	6	4	5	4
LD	3 -	5 -	3 -	3 -	1 5	3 7	3 6	1 7
M-LD	3 3	3 5	3 4	2 3	1 2	2 7	3 5	3 7
F-LD	3 3	2 3	2 5	2 3	5 6	1 3	4 6	1 1
ULD-7 pat	-	-	-	-	5	3	6	1
DZ	2 -	3 -	3 -	2 -	2 -	4 -	3 1	4 5
UDZ-22	-	-	-	-	-	-	3	5

Table 3 *3p- deletion mapping results. Depicted are the results of non-radioactive PCR analysis performed on hybrids containing the isolated der(3) chromosome of each of the five patients. The table includes the available STS and polymorphic PCR markers that have been ordered on the current consensus map of this region of 3p25. The der(3) chromosome breakpoint of MN is localised significantly distal to the 3p- breakpoints of the other four patients and MN's breakpoint lies near the 3p25.3-p26 border*

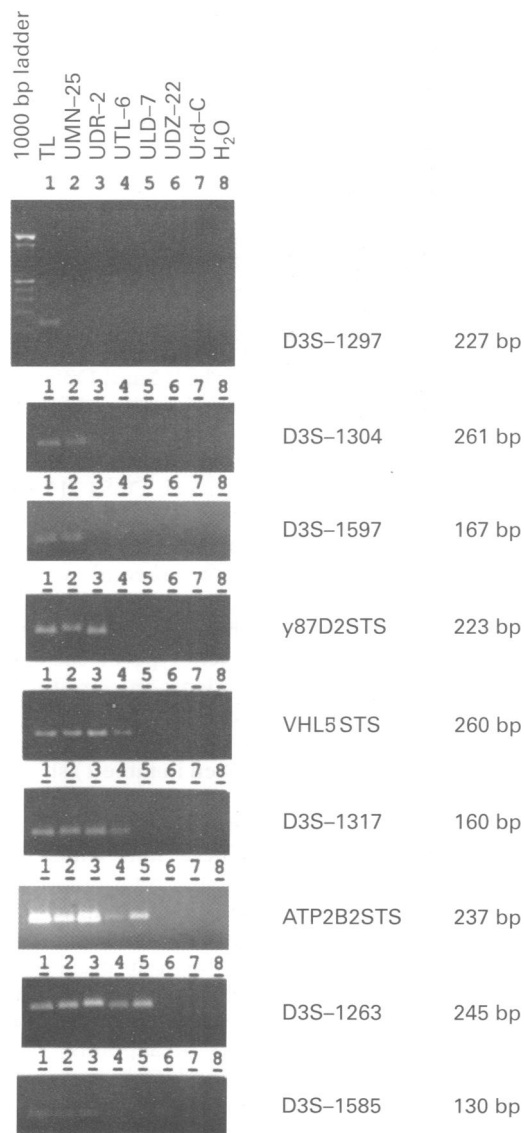|	UMN-25	UDR-2	UTL-6	ULD-7	UDZ-22
D3S1297 (3p26)	–	–	–	–	–
D3S1304	+	–	–	–	–
D3S18 (*FISH)	+	–	–	–	–
D3S1597	+	–	–	–	–
223 bp STS	+	+	–	–	–
VHL 5'	+	+	+	–	–
VHL 3'	+	+	+	–	–
D3S1317	+	+	+	–	–
D3S601/ATP2B2	+	+	+	+	–
D3S1038	+	+	+	+	–
D3S587		+		+	–
D3S1263	+	+	+	+	–
D3S1585	+	+	+	+	+
D3S1110	+	+	+	+	+
D3S1255	+	+	+	+	+
D3S1252	+	+	+	+	+
D3S1259	+	+	+	+	+
D3S1289 (3p21)	+	+	+	+	+
D3S659 (3p13)	+	+	+	+	+

Figure 1 STS analysis of isolated 3p- chromosomes. This series of ethidium bromide stained 2% agarose gels shows the results of non-radioactive PCR analysis of somatic cell hybrids containing the der(3) chromosome from each of the five patients described in the study. The results of the STS analysis using the markers found to flank each breakpoint are displayed in this figure.
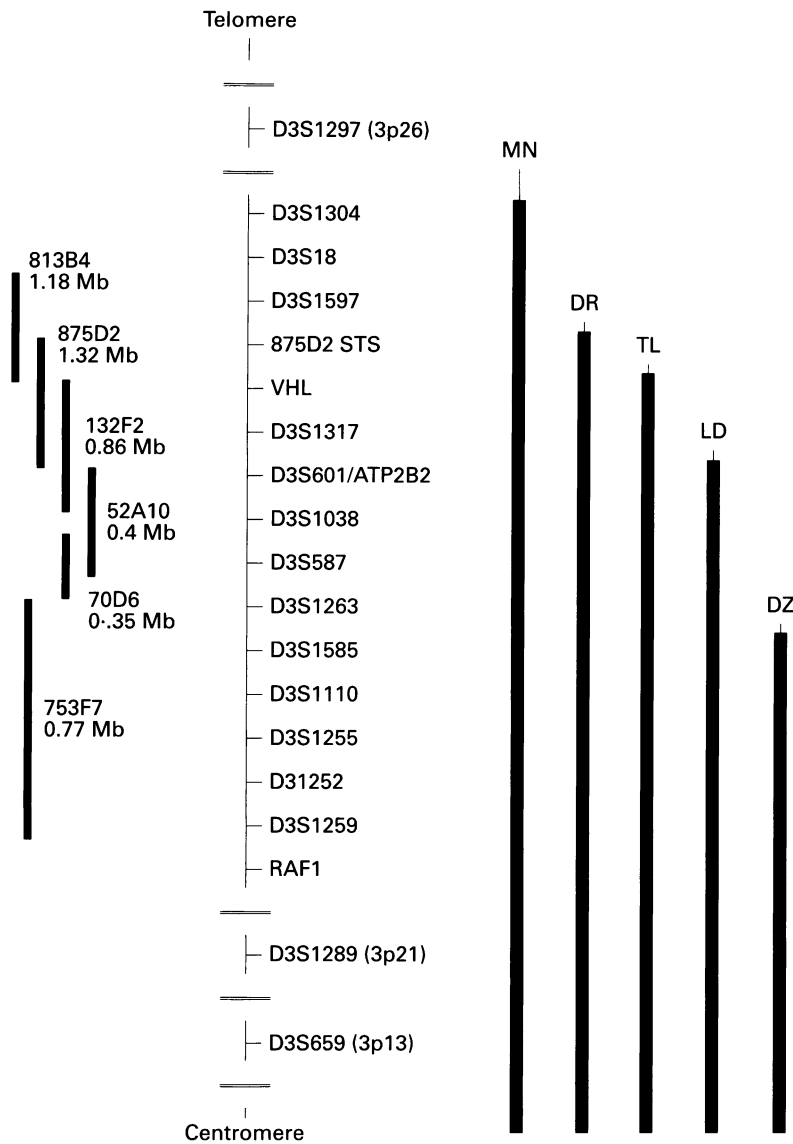
and this was confirmed by the allelotype analysis. The polymorphic markers did not yield significant information on the location of the breakpoints in these patients owing to a lack of informativeness of many of the tested markers.

ISOLATION OF DER(3) CHROMOSOMES IN SOMATIC CELL HYBRIDS
Following the fusion of lymphoblasts or fibroblasts from each of the five patients with the Urd-C mutant hamster cells and selection in medium lacking uridine, resultant hybrid lines were screened using non-radioactive PCR. We successfully isolated hybrids containing the der(3) chromosomes in the absence of the normal chromosome 3 from all five patients. Table 2 summarises the results of the polymorphic microsatellite analysis on the lymphoblast cell lines derived from each of the patients, their parents (when available), and the resulting 3p- hybrids generated.

MOLECULAR CHARACTERISATION OF ISOLATED DER(3) CHROMOSOMES
The somatic cell hybrid lines containing the der(3) chromosome from each of the five patients were tested for amplification of PCR markers from 3p. Table 3 summarises the results obtained from this analysis. The data show that the breakpoints present in the four true 3p- patients are distinct and occur over a region several megabases in size within 3p25.3. DZ had the most proximal breakpoint (between D3S1585 and D3S1263). LD had the second most proximal breakpoint localised distal to the ATP2B2 gene STS (D3S601), but proximal to D3S1317. The breakpoint in TL was localised between VHL and the 875D2 STS marker and the breakpoint in DR was localised between the 875D2 STS and D3S1597.

The der(3)t(3;?) from MN was localised between D3S1304 and the 3p26 marker D3S1297, and may actually be in 3p26, unlike the 3p25.3 breakpoints found in the four true 3p- patients. Fig 1 shows the results of a non-radioactive PCR analysis of the various 3p- hybrids with markers identified that flanked each of the respective breakpoints.

The markers used to characterise the 3p- chromosomes molecularly have been ordered relative to each other and most have been positioned on the genetic map.[17] Moreover, many of the markers have also been used to isolate YAC clones from the CEPH YAC library.[19] From the previously characterised YAC clones in this region, we were able to identify those that contain markers proximal as well as distal to the breakpoints of the 3p- chromosomes from each of the four true 3p- syndrome patients. Thus, we have identified YAC clones that presumably contain sequences crossing each of the 3p25 breakpoints from all four 3p- syndrome patients. Fig 2 summarises our analysis on the localisation of the 3p- deletion breakpoints from the five patients characterised, and shows the YAC clones that should cross the breakpoints from the four true 3p- patients.

Telomere

813B4
1.18 Mb

875D2
1.32 Mb

132F2
0.86 Mb

52A10
0.4 Mb

70D6
0·.35 Mb

753F7
0.77 Mb

|— D3S1297 (3p26)

MN

|— D3S1304

|— D3S18

|— D3S1597

|— 875D2 STS

|— VHL

|— D3S1317

|— D3S601/ATP2B2

|— D3S1038

|— D3S587

|— D3S1263

|— D3S1585

|— D3S1110

|— D3S1255

|— D31252

|— D3S1259

|— RAF1

DR

TL

LD

DZ

|— D3S1289 (3p21)

|— D3S659 (3p13)

Centromere

Figure 2   Location of 3p- breakpoints on the current 3p25 map. The 3p- chromosomes described in the text are depicted to the right of the current 3p25-p26 consensus map. The narrow bar at the telomeric end of each chromosome is meant to illustrate that the actual position of the chromosome terminus within the region defined by flanking markers is unclear. A partial contig of overlapping CEPH YAC clones is displayed on the left. The contig was constructed using information from our previous study[15] and data available from the Human Genome Center. We were able to confirm overlapping YAC sequences within the contig surrounding the VHL gene with the markers shown here. However, there is an as yet unresolved gap between overlapping YACs 52A1 and 70D5 and YAC 753F7.

Discussion

We report here the clinical features of two new 3p deletion patients. One patient has a del(3)(p25) and expresses features typical of the 3p- syndrome while the other has a more complex chromosome rearrangement, der(3) t(3;?)(p25.3;?), with concomitant clinical features that distinguish him from the classical 3p- syndrome patients. Using cosmid probes mapped to the subtelomeric region of the p arm of chromosome 3, we have shown that patients TL and DR have subtelomeric deletions. A previous study had already shown that patients LD and DZ also had subtelomeric deletions.[9] Patient MN has apparently lost subtelomeric 3p sequences on the derived chromosome 3 as the subtelomeric probe only generated a single signal against transformed lymphoblasts from this patient.

The der(3) chromosomes of all five patients were isolated from their normal chromosome 3 homologues in interspecific somatic cell hybrids and further characterised with a series of ordered 3p25-p26 PCR amplifiable markers. The breakpoints of the four 3p- patients were distributed over a region of at least 3 megabases within 3p25.3 whereas the der(3)t(3;?) of the fifth patient, MN, had a more distal breakpoint lying presumably in 3p26. Because the derived chromosome 3 in MN has a significantly more distal breakpoint and because of the widely divergent phenotypic picture present in this patient, it seems likely that his phenotype is more the result of the triplicated chromosome material of unknown origin translocated onto 3p25 rather than the deletion of 3p material distal to the 3p25-26 breakpoint.

There were two major goals of this research. The first was a high resolution molecular localisation of the breakpoints of our five 3p deletion patients on the current consensus map. Our analysis, which has precisely defined the distinct breakpoints of four del(3)(p25) chromosomes, confirms the results of Phipps *et al.*[9] In our study, patient DZ had the most proximal breakpoint localised at least 3 megabases centromeric to the breakpoint of patient DR. The breakpoint of patient TL lies just proximal to that of DR separated by a single marker, a chromosome 3 amplicon of 223 bp derived from CEPH YAC 875D2 whose order relative to other 3p25 markers is defined by these two breakpoints. The breakpoint of patient LD was previously positioned between ATP2B2 and D3S18[9] and it thus was unclear if she was deleted for the VHL disease gene. Our analysis refines the location of the 3p- breakpoint of patient LD to a region of about 200 kb between D3S1317 and the 3' telomeric end of the ATP2B2 gene, and this patient does show loss of the VHL gene.

Our second goal was to determine whether there was a relationship between the location of the 3p25 breakpoint and the resultant clinical phenotype of each of the four 3p- syndrome patients in our study. A comparison between the localised breakpoints and the clinical phenotypes of our four 3p- syndrome patients (seen in table 1) shows three different classes of phenotypic features. The first class contains phenotypic traits found in all four 3p- patients. These traits, which include growth retardation, mental retardation, an abnormal nose, low set ears, and a long philtrum presumably arise through hemizygosity of DNA sequences distal to all four breakpoints. The second class of phenotypic traits are those whose appearance seems to be independent of the location of the 3p- breakpoints. This class of traits includes clinodactyly, renal abnormalities, rocker bottom feet, seizures, rotation of the colon, a triangular face, and hearing impairment. The third class of phenotypic traits are those that are absent in patients with more distal breakpoints but present in patients with more centromeric deletion breakpoints. Patient DZ, who has the most proximal breakpoint in our group of 3p- patients, is afflicted with an endo-

cardial cushion defect not observed in the other three patients. Phipps *et al*[9] found that three of their four patients analysed (one was DZ) with more proximal breakpoints had congenital heart disease. Phipps *et al*[9] suggested that a gene involved in normal cardiac development should be contained within the interval defined by D3S1250 and D3S18 and that the plasma membrane calcium transporting ATPase isoform 2 gene (ATP2B2) would be an excellent candidate gene. Our analysis has refined the location of this putative cardiac developmental gene to a much smaller region bordered by D3S1585 and D3S1317. The ATP2B2 gene is located within these boundaries and remains an excellent candidate. However, another gene characterised by us, Sec13R,[20] which is the human homologue of the yeast Sec13 gene involved in vesicle formation, is also contained within this interval and could also be considered a viable candidate gene.

Two additional phenotypic traits, a thin upper lip and micrognathia, were present in patients with more proximal breakpoints but absent in patient DR who had the most distal 3p- breakpoint. Since the breakpoint in patient TL has been positioned centromeric to the 3p- breakpoint of DR, the interval between these two breakpoints may contain a gene responsible for normal craniofacial development. However, we realise that this analysis is only based on a very small number of 3p- patients. The molecular analysis of additional 3p- syndrome patients should help us to determine if some of our potentially interesting results are real, both with respect to the endocardial cushion defect, and to the thin upper lip and micrognathia.

The isolation of the der(3) chromosome from each of the five patients in our study within somatic cell hybrids made all markers from 3p25-pter informative for the molecular localisation of the respective breakpoints. The hybrids form an excellent 3p25 deletion mapping panel that will allow for the rapid localisation of markers to this region. The panel of hybrids should also aid in the localisation of genes to this region of chromosome 3 that can be considered candidate developmental genes responsible for some of the phenotypic traits observed in patients with the 3p-syndrome.

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