An interstitial deletion of 6p24-p25 proximal to the *FKHL7* locus and including *AP-2α* that affects anterior eye chamber development.

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Abstract

The *FKHL7* gene has been implicated in the pathogenesis of glaucoma/autosomal dominant iridogoniodysgenesis (IGDA) (*IRID1*). This has been supported by mutations in some glaucoma and IGDA patients and the development of anterior eye chamber anomalies in patients with 6p deletions affecting the 6p25 region. We report a case with anterior eye chamber anomalies and an interstitial deletion of 6p24-p25 that does not include the *FKHL7* gene, suggesting the possible additional involvement of another locus, within 6p24-6p25, in anterior eye chamber development. A candidate gene is *AP-2α*, which is contained within the deleted segment and plays a role in anterior eye chamber development.

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Deletions affecting the 6p25 region of chromosome 6 have received a lot of attention recently owing to the identification of the *FKHL7* (*FREAC3*) gene within that region and the correlation of mutations at that locus with anterior eye chamber anomalies including glaucoma and autosomal dominant iridogoniodysgenesis (IGDA).[1 2] This gene is a member of the forkhead/winged helix transcription factor family and its murine homologue *Mf1* is expressed during the development of the brain, the skeletal system, and the eye. A homozygous deletion of this gene in the mouse leads to the development of congenital hydrocephalus and anterior eye chamber anomalies.[2] The phenotypes of 6p deletion cases where deletions of this locus have been shown agree with the mouse model.[2] The eye phenotypes are variable and include corneal opacities, iris coloboma, and Rieger type anomalies.[3 4] However, although mutations in the human gene have been found in patients with glaucoma or IGDA, it has recently been reported that no mutations have been detected in a subset of glaucoma/IGDA patients belonging to 6p25 linked families.[5 6] These reports suggest that it is possible that another locus within 6p25 is involved in anterior eye chamber development.

We present a case with microphthalmia and corneal clouding and an interstitial deletion of 6p, in which the 6p24.2-p25 region is deleted but not the distal part of 6p25 that contains the *FKHL7* gene. This may have implications for the localisation of another gene involved in anterior eye chamber development.

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Materials and methods

Fresh blood was obtained and metaphase chromosome spreads were prepared by standard techniques.

FLUORESCENT IN SITU HYBRIDISATION (FISH)

Cosmid DNA was cultured, prepared, and purified by standard techniques. YAC clones were not isolated from endogenous yeast DNA before FISH; the total yeast DNA was prepared as described previously.[7] PAC DNA was prepared as recommended by the MRC HGMP Resource Centre and BAC DNA as recommended by Genome Systems. All clones were labelled with biotin-14-dATP or digoxigenin-11-dUTP by nick translation (Bio-Nick Labeling System or Nick Translation System respectively, BRL Life Technologies, USA).

In situ hybridisation was performed as previously described.[7] Briefly, probes for each slide were combined as required (50 ng of YAC per slide or 100 ng of cosmid, BAC, or PAC per slide), dried down, and suspended in 50% formamide, 1% Tween-20, 20% dextran sulphate along with salmon sperm DNA (100 × w/w) and Cot-1 DNA (50 × w/w). The probe mixes were then denatured by heating to 75°C for three minutes, prehybridised for 30 minutes, and applied to the slides, which had themselves been denatured by treating in 70% formamide, 2 × SSC for 2.5 minutes at 65°C. Hybridisation was carried out at 37°C for 16 hours. Post-hybridisation washes were 50% formamide, 2 × SSC for 15 minutes at 45°C followed by 0.1 × SSC for 15 minutes at 60°C and 4 × SSCT for five minutes at room temperature. Signals from biotin labelled probes were developed using alternate layers of avidin-fluorescein-isothiocyanate (avidin-FITC) and biotinylated anti-avidin. Those from digoxigenin labelled probes were developed with a layer of sheep antidigoxigenin conjugated to tetramethylrhodamine-isothiocyanate (TRITC-antidigoxigenin) followed by one layer of donkey anti-sheep-TRITC. Slides were mounted in Vectashield antifading medium (Vector Laboratories, USA) containing 80 ng/ml 4',6-diamidino-2-phenylindole (DAPI) as counterstain.

Signals were visualised under a Zeiss Axioplan microscope equipped with a cooled charge coupled device (CCD) camera (Photometrics, USA) and Smartcapture image analy-

Location	Probe	BT	Markers
6p25.3	c6K23	+	
6p25.3	790-h-7	+	D6S1600
6p25	947-d-4	+	D6S344
6p25	905-f-3	+	WI-9400
6p25	870-d-6	+	**FKHL7**
6p25	954-h-10	+	GMDS
6p25	B19	+	P16
6p25	B20	+	P19
6p25	952-h-4	+	D6S1713
6p25	814-d-12	+	D6S1617
6p25	878-b-10	–	D6S477
6p25	860-h-6	–	F13A
6p25	853-c-3	–	F13A
6p25	938-b-10	–	PRBE1
6p25	dJ29L9	–	DSP
6p25	963-h-4	–	BMP6
6p25	887-h-3	–	D6S309
6p24.3	848-c-12	–	D6S410
6p24.3	808-a-10	–	
6p24.3	826-a-12	–	D6S470
6p24.3	886-c-1	–	AP-2
6p24.2	938-d-8	–	AP-2
6p24.2	cB11.7	–	AP-2
6p24.2	897-f-9	+	D6S202
6p24.1	915-e-8	+	GCNT2
6p24.1	933-c-3	+	EDN1
6p24.1	961-e-7	+	ZNF40
6p24.1	844-h-3	+	ZNF40
6p24.1	886-a-2	+	D6S429
6p24.1	779-d-5	+	D6S338
6p23	930-d-2	+	JMJ
6p23	912-g-9	+	
6p23	917-a-1	+	D6S285
6p22.3	738-b-5	+	D6S422
6p22.2	p999b	+	SOX4

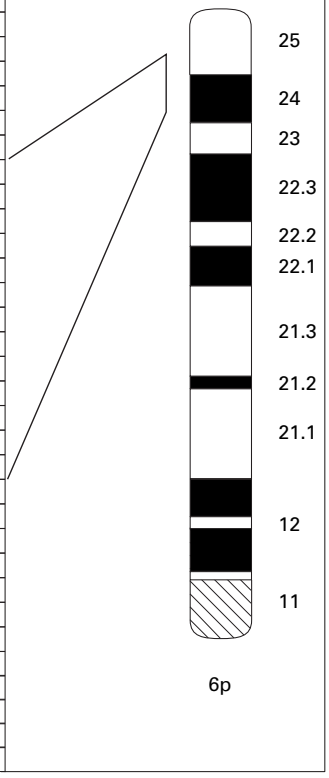

Figure 1 FISH analysis of proband. The hybridisation (+) or failure of hybridisation (–) of probes to the derivative chromosome 6 is shown. Probes are listed according to their order on 6p from distal (top) to most proximal (bottom). B19 and B20 are overlapping BAC clones containing PI6 and PI9 respectively, cosmid clones have the prefix "c", and the PAC clone has the prefix "dƒ". The deleted region is highlighted in grey. Markers contained within each particular clone are shown to the right. The FKHL7 gene, implicated in anterior eye anomalies, is highlighted in bold. An ideogram of chromosome 6p indicating the deleted region is shown to the right.

sis system (Vysis, UK). G banding was enhanced during image analysis. At least 10 metaphase cells were examined in order to confirm the results.

CLONE DETAILS

In total, 30 YAC clones, one PAC, two BAC, and two cosmid clones mapping to defined positions on 6p[4] were used (fig 1).

Case report

The patient was the first child of healthy, non-consanguineous parents. At 32 weeks' gestation intrauterine growth retardation was detected and he was born at 36 weeks' gestation by normal delivery with a birth weight of 3126 g. At birth dysmorphic features were noted and he had mild respiratory distress.

At 2 weeks of age he was noted to have hypertelorism, short palpebral fissures, and left microphthalmia with corneal clouding, which were the only ocular findings. His ears were abnormal with crumpled pinnae and he had a small jaw and a rather flat nose. A systolic murmur was audible at the left sternal edge and he had striking pectus excavatum. He had very thin limbs and positional talipes calcaneovalgus. His long thumbs were proximally placed and the thumb on the right appeared to be floating with a contracture at the intraphalangeal joint on that side.

Subsequent cardiac investigations showed an atrial septal defect and ventral septal defect for which he had corrective surgery. He also required the insertion of a pacemaker. After the operation his weight gain improved. At 1 year 11 months the patient has progressed developmentally, has some single words, and can walk.

Cytogenetic analysis showed a karyotype of 46,XY,del(6)(p23p25). Both parents had normal chromosomes suggesting that the deletion occurred de novo.

Results

The cytogenetic diagnosis was confirmed by FISH using 35 probes mapping to the 6p22.2-pter interval (fig 1). This experiment confirmed the presence of an interstitial deletion as follows. All probes mapping to 6p22.2-6p24.1 hybridised to the del(6) chromosome and the proximal deletion breakpoint was found to be at the YAC clone 897-f-9 mapping in 6p24.2 (fig 1). The YAC clone 938-d-8 failed to hybridise on the del(6) chromosome and since it is located distal to but overlapping 897-f-9, the breakpoint can be localised within this region. Since these YAC clones contain the *AP-2α* gene, the deletion of *Ap-2α* was confirmed by using the cosmid B11.7 that contains this locus. The distal breakpoint has been localised within the proximal part of 6p25 between the YAC clones 878-b-10 which failed to hybridise on the del(6) and 814-d-12 that did hybridise on the del(6) and maps distal to 878-b-10 (fig 1). The cytogenetic finding could thus be refined to 46,XY,del(6)(p24.2p25).

In order to verify the distance between *FKHL7* and the distal breakpoint, we used a tilepath of YAC and BAC clones. The *FKHL7* gene is contained within YAC 870-d-6 and at the distal end of YAC 954-h-10 (fig 2). A cluster of protease inhibitor genes (*PI6*, *PI9*, and *ELANH2*), covering a 200 kb genomic segment, map centromeric to the *FKHL7* gene; this cluster, including the marker D6S1338, is contained within YAC 927-c-12 which has a length of 1200 kb.[8] This YAC does not contain D6S344 (towards the telomere) or D6S1713 (towards the centromere, fig 1). The entire protease inhibitor cluster is present on the del(6) since the two BAC clones (B19 and B20) that cover this region and the corresponding YAC clones all hybridised to the del(6) (fig 1) . YAC 814-d-12 (1000 kb) does not overlap 927-c-12 and hybridises on the del(6); in contrast clone 878-b-10 that maps immediately centromeric to it is deleted (fig 2). Therefore the distal deletion breakpoint must be located within YAC 814-d-12. The minimal distance between the breakpoint and *FKHL7* can be calculated as the length of 927-c-12, which does not overlap 814-d-12 and would be 1200 kb (fig 2).

Discussion

Deletions of 6p have been important in confirming the role of the *FKHL7* gene in embryonic development. Although mutations

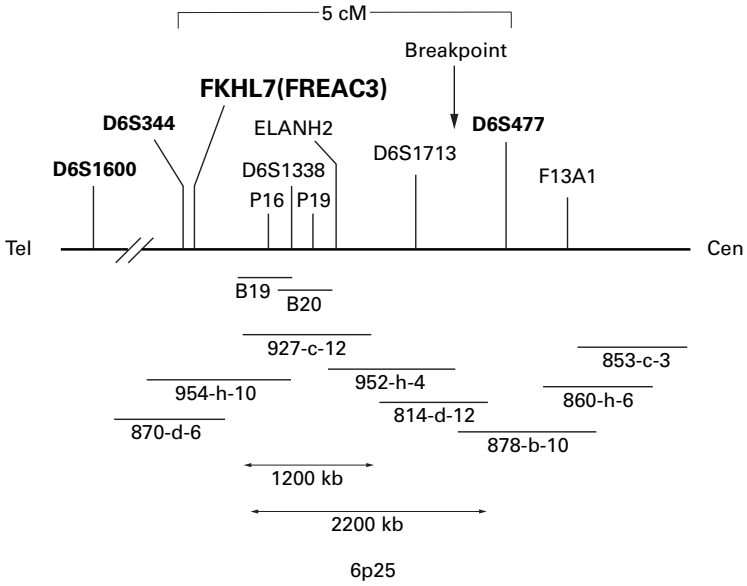

Figure 2 Physical map of proximal 6p25. The YAC contig covering the region FKHL7 to F13A that has been used is shown (data taken from references 1 and 8, the WIGR July 1997 data release, and this study). The minimal and the possible maximal distance between the distal deletion breakpoint and FKHL7 is shown below. Markers D6S344 and D6S477 define the original genetic interval (5.0 cM) for IGDA.[9] Markers D6S1600 and D6S344 mark the position of a second possible locus.[5]

in this gene are associated with glaucoma/anterior eye chamber anomalies, they have not been detected in all disease families linked to 6p25. The genetic interval containing the candidate gene(s) for glaucoma/IGDA (*IRID1*) was originally defined as being between D6S344 and D6S477,[9] but subsequently one possible additional locus has been assigned between D6S1600 and D6S344 (fig 2) using data from two out of four families with no *FKHL7* mutations.[5] Since the distal deletion breakpoint in the patient described in this study is located at least 1200 kb proximal to *FKHL7*, a possible third locus may be located in the region around D6S477 (fig 2). The possibility of a position effect on the expression of *FKHL7* cannot be entirely excluded, but the minimal distance between breakpoint and gene of 1200 kb provides a substantial buffer against effects from elements located within the proximal part of 6p24. The detection of small inversions between the distal breakpoint and *FKHL7* would be beyond the limits of resolution of metaphase FISH and, although unlikely, this possibility cannot be excluded.

The effect of the hemizygosity of this putative "anterior eye chamber anomaly" locus seems to be at the threshold of being able to cause a defect, since only one of the patient's eyes was affected.

Another possible explanation for the eye defects can be given through the deletion of the *AP-2α* gene. It has been shown in two recent studies involving chimeric mice that hemizygosity or lower than normal levels of *AP-2α* expression can cause microphthalmia and other anterior eye chamber anomalies including corneal clouding.[10 11] Since the patient is hemizygous for this gene, the human eye phenotype is consistent with the eye phenotypes observed in the mouse models. This conclusion is also consistent with another published case of an interstitial deletion of 6p, reported as involving the region 6p22-p24 and associated with sclerocornea.[12]

The small jaw and limb abnormalities observed in our patient correlate with the deletion of the *AP-2α* gene and its demonstrated role in craniofacial and limb development.[13 14] Another interesting aspect of the phenotype is the coincidence of pectus excavatum with the deletion of the *BMP-6* gene. *BMP-6* is expressed in the developing sternum and *BMP-6* null mice show delayed ossification of this bone.[15]

In summary, the phenotypic characteristics of this patient (hypertelorism, anterior eye chamber anomalies, abnormal ears, heart defects, and mild developmental delay) constitute part of the 6p terminal deletion syndrome.[3 4] The patient does not yet show any signs of hearing loss, which is the additional characteristic of all 6p terminal deletion cases where the deletion of the *FKHL7* locus has been reported.[2 4] It is possible that either the hearing defect is directly linked to hemizygosity of *FKHL7* or that the hearing defect is the result of another locus contained within the segment of 6p25 which is not deleted in the case presented here.

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