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 iridogoniodygenesis (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 iridogoniodygenesis (IGDA). 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. The phenotypes of 6p deletion cases where deletions of this locus have been shown agree with the mouse model. The eye phenotypes are variable and include corneal opacities, cataracts, and Rieger eye abnormalities. 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. 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.

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. 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. 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 Axiosplan microscope equipped with a cooled charge coupled device (CCD) camera (Photometrics, USA) and Smartcapture image analy-
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 “p”. 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 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-2 gene, the deletion of AP-2 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 tiling path of YAC and BAC clones. The FKHL7 gene is contained within YAC 870-d-10, which maps at the distal end of YAC 870-d-6 and is deleted within 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. 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).

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

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. 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.

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. 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. 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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