Agenesis of the corpus callosum with Probst bundles owing to haploinsufficiency for a gene in an 8 cM region of 6q25

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Abstract

Agenesis of the corpus callosum (ACC) is a relatively common brain abnormality resulting from developmental defects either limited to the structures leading to the proper formation of the corpus callosum or involving the embryo forebrain more generally. ACC is genetically heterogeneous with autosomal dominant, autosomal recessive, and X linked inheritance and has also been reported in subjects with aneuploidies involving several chromosomes. Among them, distal 6q deletions have been consistently reported in association with ACC, suggesting that there is a gene in the deleted region whose haploinsufficiency impairs normal corpus callosum development. We have studied a child with ACC with Probst bundles and a deletion at 6q25 of about 8 cM, from D6S1496 to D6S437. Probst bundles are the axons that should have formed the corpus callosum but, unable to cross the midline owing to absence of the massa commissuralis, they run longitudinally along the medial walls of the lateral ventricles from the frontal to the occipital lobes. Thus, their presence suggests that a gene located in the 6q deleted region is specifically involved in the formation of the massa commissuralis and that its haploinsufficiency leads to primary ACC.

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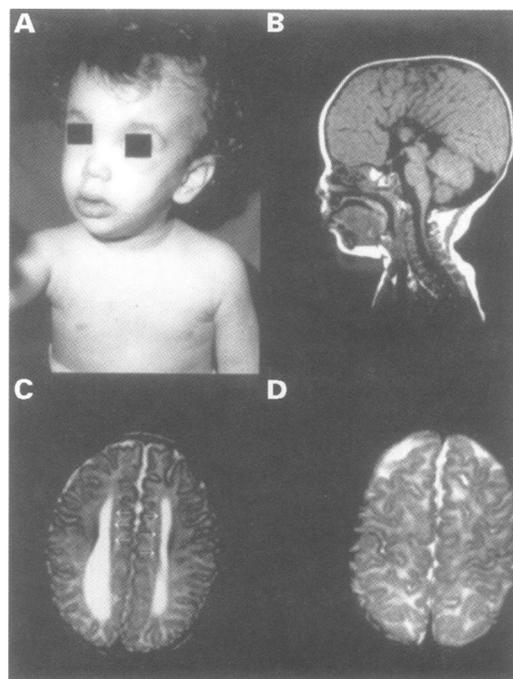B Pirola
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In reviewing the genetics of agenesis of the corpus callosum (ACC), Dobyns[1] concluded that aneuploidies (deletion or duplication) for no less than 19 chromosome regions are consistently associated with ACC. Thus, all these regions are likely to harbour genes that act in a dominant way interfering, by haploinsufficiency or excessive dosage, with the development of the corpus callosum. We studied a child whose brain abnormalities were detected during routine prenatal echography. Cytogenetic analysis performed at birth showed an interstitial deletion around 6q25. Since haploinsufficiency for this region is consistently associated with ACC,[1] we decided to define the deletion at the molecular level.

Case report

The female patient was born at 38 weeks of gestation to a primiparous, 33 year old, healthy woman and an unrelated, 41 year old, healthy father.

Fetal ultrasonography in the last trimester of gestation showed enlargement of the cerebral ventricles (hydrocephalus). Delivery was by caesarean section. Birth weight was 2600 g (3rd-10th centile), length was 47 cm (3rd-10th centile), and head circumference (OFC) was 32.5 cm (−2 SD). Apgar scores were 3 at one minute and 8 at five minutes. Dysmorphic features included dolichocephaly, high arched and cleft palate (occult submucous), low set ears of normal size, hands with relatively long distal phalanges, and an anteriorly placed anus. Marked muscular hypotonia was also noted which led, in conjunction with the cleft palate, to feeding problems which were overcome at 5 months.

The ocular fundi were normal. CT scan of the head showed ACC and dilatation of both lateral ventricles. At the age of 5 months the patient showed moderately delayed psychomotor development and mild lateral nystagmus. Magnetic resonance imaging (MRI) confirmed ACC and led to detection of Probst bundles (fig 1B, C, D). At the age of 9 months (fig 1A), her weight (6200 g) and height (66 cm) were below the 3rd centile; head circumference was 44 cm (~50th centile). Psychomotor retardation (mild to moderate), hypotonia, and nystagmus were still present.

Cytogenetic and molecular studies

Cytogenetic analysis of cultured peripheral lymphocytes was performed with standard high resolution techniques. QFQ and RBA banding (more than 550 bands) showed an unbalanced karyotype with an interstitial deletion of the long arm of chromosome 6 at the level of q25 (fig 2A).

Both parents had a normal karyotype. FISH[2] with a set of YACs covering the deleted region, selected according to the Genome Database (www-genome.wi.mit.edu/cgi-bin/contig/phys_map) allowed the proximal and distal breakpoints to be identified. The results of the FISH analysis are summarised in table 1 and in fig 2B, C.

The deletion of 8-9 cM extended from locus D6S1496 (154 cM) to D6S437 (162 cM). Accordingly, the final karyotype was: 46,XX,del(6)(q25.1q25.3) de novo. Parental origin of the deletion was determined by PCR

Figure 1   (A) The patient at the age of 9 months. (B) T1 weighted sagittal image through the midline. The corpus callosum and the cingulate gyrus are absent. The medial hemispheric sulci are running all the way towards the third ventricles. (C) T2 weighted axial image showing dilatation of the atria and occipital horns of both lateral ventricles (colpocephaly) because of lack of the corpus callosum. Arrows indicate Probst bundles. (D) T2 weighted axial image showing a normal cortical gyral pattern.

Table 1   FISH results: locus copy number on the abnormal chromosome 6

Locus	Map position (cM)	YAC	Signal
D6S472	133	970A12	+
D6S314	144	857D11	+
D6S292	146	915F1	+
D6S311	148	800H6	+
D6S1654	151	972A3	+
D6S1496	154	806B12	+
D6S440	155	767F3	−
D6S290	155	771C12	−
D6S415	161	830B11	−
D6S437	162	845F6	+

+ and − refer to the presence or absence of FISH hybridisation signals on the abnormal chromosome 6.

Discussion

Agenesis of the corpus callosum, either isolated or associated with other malformations, is among the most common developmental brain abnormalities.[4][5] Embryological studies suggest that there are two primary and two secondary types of callosal abnormality.[1] Primary forms include (1) defects in which axons form but are unable to cross the midline because the massa commissuralis is absent. In this case longitudinal fibres that should have formed the corpus callosum, known as Probst bundles, run longitudinally along the medial walls of the lateral ventricles from the frontal to occipital lobes; (2) defects in which the commissural axons or their parental cell bodies fail to form in the cerebral cortex.

Secondary callosal abnormalities include (1) absence of the corpus callosum associated with major malformations of the embryonic forebrain (such as encephaloceles and holoprosencephaly) originating before the formation of the anlage of the corpus callosum; (2) degeneration or atrophy of the corpus callosum, leading to its extreme thinning, which may be

analysis of CA repeats[3] (fig 3A). Probe D6S1633, localised within the deleted region, detected a polymorphism informative for a paternal origin of the deletion. Paternity was confirmed by PCR analysis of CA repeats of loci D2S405 (fig 3B) and D15S1048.
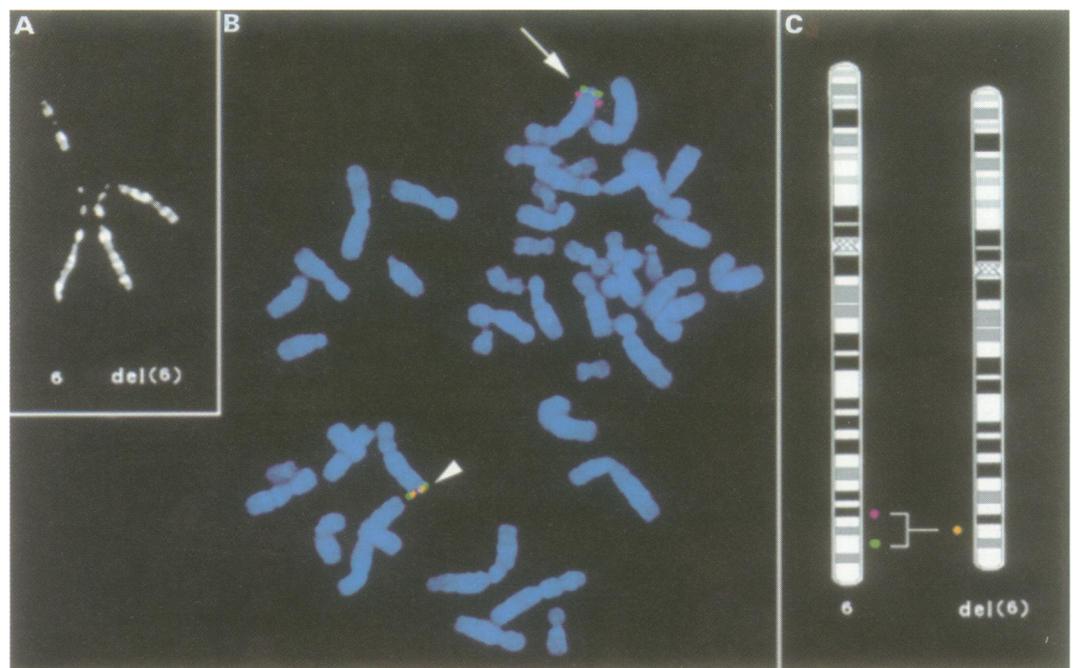
Figure 2   (A) Chromosomes 6 from the patient. (B) Metaphase from the patient after FISH with YAC 806B12 (D6S1496, 154 cM) in red and with YAC 845F6 (D6S437, 162 cM) in green. Arrow indicates normal chromosome 6, arrowhead indicates the deleted one with fusion of colours. (C) Ideogram of the normal and the abnormal chromosomes 6. Colours refer to FISH hybridisation signals.

Figure 3 (A) PCR analysis of CA repeats at locus D6S1633. The patient has only one allele, inherited from her mother. (B) PCR analysis of CA repeats at locus D2S405. The patient is heterozygous having received one allele from each parent. Arrows indicate paternal and maternal alleles.

region in 6q25 in which a gene responsible for the development of the corpus callosum should be located. The corresponding physical distance cannot be precisely estimated since the deleted region is not covered by a complete YAC contig. The longitudinal bundles of Probst represent axons which, unable to cross the interhemispheric fissure, have diverted 90° posteriorly to run parallel to the interhemispheric fissure, medial to the medial walls of the lateral ventricles of their hemisphere of origin.[14] [15] The bundles of Probst can therefore be considered a marker for distinguishing true callosal agenesis from secondary callosal hypoplasia or atrophy. Their presence could indicate that in our patient the ACC gene located in the deleted region is specifically involved in the formation of the massa commissuralis, since its haploinsufficiency would make the axons unable to cross the midline. Since there are no studies on the parental origin of the other cases with del(6q) and ACC, we do not know if the ACC-6q gene is imprinted as is the case for the transient neonatal diabetes gene in 6q22-q23.[16]

mistaken for true agenesis. ACC has been observed in families with autosomal dominant, autosomal recessive, and X linked inheritance,[1] [6] but it is also associated with several chromosome rearrangements including 6q distal deletions.[1] The few genes involved in the development of the corpus callosum that have been isolated interfere with brain development in general since their mutations lead to ACC associated with malformations such as lissencephly and holoprosencephaly.[7] [8] At present, L1CAM is the only gene whose mutations, generally responsible for a wide spectrum of neurological abnormalities, result in primary ACC without other congenital anomalies in a few males.[9] This study suggests that in the 6q region deleted in our patient a gene is located whose haploinsufficiency results in primary ACC. Other published reports confirm this relationship. ACC was found at necropsy in a 21 week male fetus with del(6)(q23-qter)[10] and, using transfontanellar ultrasound, in a newborn male with del(6)(q25.1-q27).[11] Similarly, ACC was found on studying the brains of two newborns, both with a r(6) (cases 1 and 4[12]), in which part of the distal short and long arms had probably been lost in the formation of the ring. No ACC was reported in case 2 of Narahara *et al*[13] with a 6q deletion identical to that of our case. This indicates either that the two deletions are different in spite of the same cytogenetic interpretation or that ACC has incomplete penetrance. In all these cases other malformations were reported, possibly linked to haploinsufficiency for other genes in the deleted region. We thus defined a 8-9 cM

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