Pre- and Postnatal Analysis of Chromosome 1q44 Deletion in Agenesis of Corpus Callosum

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Key Words

AKT3 · Agenesis of corpus callosum · Deletion 1q44

Abstract

Agenesis of corpus callosum (ACC) is one of the common brain abnormalities and also a common finding in children with mental disability. ACC is heterogeneous and can occur as an isolated condition or as part of a syndrome. ACC can be accurately identified by the absence of the cavum septum pellucidum and tear drop effect of the lateral ventricle after 18 weeks of pregnancy in an ultrasound scan. Genetic causes have been attributed to 30–45% of cases with ACC. Submicroscopic deletions of 1q43q44 have been reported in several cases of ACC. The *AKT3* gene, mapped to 1q44, is required for the development of the callosum and brain size. It is considered to be a candidate gene for ACC. We studied a total of 22 cases with ACC, in pre- and postnatal samples using FISH probes. None of the samples showed a deletion in 1q44, implying that the *AKT3* gene may not be associated with ACC.

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Agenesis of corpus callosum (ACC) is a congenital abnormality in which there is a partial or complete absence of the corpus callosum. It connects the 2 cerebral hemispheres in the brain. The disruptions to the development of the corpus callosum occur during the 6–20th week of

pregnancy. ACC is one of the most frequent malformations in the brain with a reported incidence ranging between 0.5 to 70 per 10,000 births for the general population and ~2% of those with mental disabilities [Schell-Apacik et al., 2008].

ACC can be diagnosed by CT scan or MRI and during pregnancy by routine prenatal ultrasound. ACC can be accurately identified by targeted examinations performed at 18–20 weeks or later. Failure to visualize the cavum septum pellucidum beyond 18 weeks' gestation may suggest ACC. Fetuses with callosal agenesis were likely to be born 3–4 times more frequently prior to 37 weeks' gestation [Glass et al., 2008].

The primary function of the corpus callosum is to transfer and integrate motor, sensory, and cognitive performances between the 2 hemispheres of the brain. The most frequent clinical findings in patients with ACC are mental retardation (60%), visual problems (33%), speech delay (29%), seizures (25%), and feeding problems (20%). Some cases with no developmental delay and normal intelligence have also been reported [Schilmoeller and Schilmoeller, 2000; Schell-Apacik et al., 2008].

A prospective study by Moutard et al. [2003] showed that nearly 80% of children are associated with a normal IQ. However, with age, the IQ tends to deteriorate. In their study, Volpe et al. [2006] showed that the outcome of isolated partial ACC was not better than that of complete ACC.

Table 1. Types of samples included in the study

Sample	Number (n = 22)
Amniotic fluid	3
Cord blood	7
Peripheral blood	12

Fig. 1. FISH study showing a normal signal (Spectrum Orange) pattern for RP11-384H20 (AKT3).

ACC is a clinically and genetically heterogeneous condition, which can be observed either as an isolated condition or as a manifestation in the context of a congenital syndrome such as Aicardi syndrome, Acrocallosal syndrome, Andermann syndrome, Marden-Walker syndrome and cerebro-oculo-facio-skeletal syndrome [Blum et al., 1990].

Submicroscopic deletions of 1q43q44 have been reported in several cases of ACC. The *AKT3* gene mapped to 1q44 is required for the development of the callosum and brain size. It represents an important candidate gene for ACC. We therefore studied a total of 22 cases with ACC in pre- and postnatal samples to identify the status of the *AKT3* gene.

Materials and Methods

Sample Details

We studied a total of 22 patients with ACC (10 prenatal and 12 postnatal samples) (tables 1–3). Peripheral blood was used for the analysis in postnatal samples. Amniotic fluid and cord blood was used in prenatal samples.

Karyotyping

All pre- and postnatal samples were cultured and harvested. The chromosome slides were prepared and banded using standard protocols [Hungerford, 1965; Seabright, 1971; Priest and Rao, 1997]. Twenty well-spread and well-banded metaphases were captured, of which 15 metaphases were screened, and 6–8 metaphases

were karyotyped using Olympus BX51 (Japan) and analyzed using Applied Spectral Imaging-Band view version 5 software, Israel. The chromosome abnormalities were identified and designated as per ISCN [2013] nomenclature.

Fluorescence in situ Hybridization

FISH analysis was used to precisely define the 1q deletion break point. The BAC clone localized to 1q44 was selected from the human DNA library in the UCSC genome browser and provided by BACPAC Resources (<http://bacpac.chori.org/home.htm>). The BAC clone RP11-384H20 (AKT3) ~180–200 kb was used.

DNA was isolated from cultured BACs, amplified and then labeled with Digoxigenin 11-dUTP by nick translation according to standard protocols. FISH probes were hybridized onto the slides and detected using anti-DIG antibody tagged with Rhodamine [Sambrook and Russell, 2001]. The probe and prepared slides were denatured, hybridized, washed, and counter stained. Slides were observed under an Olympus BX60 fluorescence microscope using a multi-band pass filter [Pinkel et al., 1988; Ried et al., 1992]. Analysis of the slides was done using the Applied Spectral Imaging version 5.0 software. A minimum of 10–15 metaphases were analyzed and a minimum of 3 metaphase pictures were captured.

Results

All the samples were negative for 1q44 probes (fig. 1). No deletion was seen for the *AKT3* gene (1q44). In this study, 45% of the samples were prenatal, and the remaining 55% of the samples were postnatal. Among the prenatal samples, cord blood formed the majority with 70% followed by 30% of amniotic fluid. Consanguinity was seen in 13.6% of ACC patients. None of the mothers with advanced maternal age had a child with ACC. In fact, the majority (91%) of mothers who had a child with ACC were in the age group of 21–30 years. In this study, 8/22 (36%) had only a corpus callosal abnormality, while 11/22 (50%) had other central nervous system (CNS) abnormalities, and 4/22 (18%) had non-CNS features in addition to ACC. To the best of our knowledge, this is the first pre- or postnatal submicroscopic deletion study for ACC from India.

Discussion

ACC is a heterogeneous condition and only 30–45% of the cases have identifiable causes. Approximately 10% have chromosomal anomalies, and the remaining 20–35% have recognizable genetic syndromes [Bedeschi et al., 2006].

ACC has been observed in constitutional trisomy as well as in some consistent chromosomal rearrangements,

Table 2. Clinical profile of prenatal ACC cases included in the study

Sample No.	Mother's age, years	Gestational age, weeks	Sex	Consanguinity	Indications
1	22	20	M	no	ACC
2	24	22	F	no	ACC, DWM
3	21	22	F	yes	ACC, semilobar holoprosencephaly, hypertelorism
4	26	20	F	no	ACC, hydrocephalus, VSD, duodenal atresia
5	26	27	M	no	ACC, ventriculomegaly
6	27	20	M	no	ACC, IUGR, micrognathia, rocker bottom feet
7	26	31	F	yes	ACC, microcephaly, IUGR, micrognathia
8	25	22	M	no	ACC
9	26	23	F	no	ACC
10	28	20	M	no	ACC, ventriculomegaly, hypertelorism, low-set ears

DWM = Dandy-Walker malformation; VSD = ventricular septal defect; IUGR = intrauterine growth retardation. All karyotypes and FISH 1q were normal.

Table 3. Clinical profile of postnatal ACC cases included in the study

Sample No.	Age	Mother's age, years	Sex	Consanguinity	Indications
11	1 day	26	M	no	ACC, EIF
12	4½ ys	38	F	no	HCC, microcephaly, DWM
13	3 ys, 5 mo	25	F	yes	HCC, cerebral atrophy
14	6 ys	33	M	no	HCC, cystic encephalomalacia, arachnoid cyst
15	2 ys	26	M	no	HCC, pachygyria, cerebral atrophy, developmental delay, dysmorphic features
16	2 ys, 4 mo	28	M	no	HCC
17	4 ys, 5 mo	35	M	no	ACC, microcephaly, f/h/o ACC
18	2 mo	26	M	no	ACC, hydrocephalus, developmental delay
19	3½ mo	25	F	no	ACC, microphthalmia, dysmorphic features
20	5 days	26	F	no	ACC, b/l ventriculomegaly, prominent 3rd ventricle, partial ACC
21	6 mo	26	M	no	
22	7 mo	29	F	no	HCC, microcephaly, developmental delay

DWM = Dandy-Walker malformation; EIF = echogenic intracardiac foci; HCC = hypoplastic corpus callosum; mo = months; ys = years. All karyotypes and FISH 1q were normal.

such as del(6)(q23), dup(8)(p21p23), dup(11)(q23qter), del(X)(p22), Wolf-Hirschhorn syndrome, and trisomy 8, 13 and 18. Other regions in which cytogenetic abnormalities have been associated with ACC include del(1)(q44), del(2)(q14), dup(5)(p15.3p13.1), dup(6)(p25), dup(14)(q23q24), del(15)(q13), and del(21)(q11q22.1) [Dobyns, 1996]. O'Driscoll et al. [2010] studied 374 individuals with ACC and structural chromosomal rearrangement and identified 12 loci consistently associated with ACC and 30 other loci likely to cause ACC. ACC-associated loci were deletions of chromosome 1p36, 1q43q44, 4p16, 6q26q27, 13q32.3q33.1, 14q12q13, 14qter, 21q22, Xp22.3,

and duplication of chromosome 8p22p21.3, 11q25, 13q34, 21q22, and Xp27.3q28.

The *AKT3* gene (OMIM 611223; v-akt murine thymoma viral oncogene homolog 3 gene) codes for a serine/threonine protein kinase which is a regulator of cell signaling in response to insulin and growth factors. Submicroscopic deletions of 1q43q44 have been reported in a variety of developmental abnormalities of the brain, including microcephaly and ACC, which also harbors *AKT3*. The AKT family of protein kinases has 3 isoforms, AKT1, AKT2 and AKT3. AKT1 and AKT2 are required for normal growth and metabolism, while AKT3 does not

appear to contribute significantly to the maintenance of a normal metabolism, but is critical for the attainment of normal organ size. However, *AKT3* exclusively controls the mass of the mouse brain by influencing both cell size and number, at least in part, through the selective activation of downstream effectors in the mTOR pathway [Nakatani et al., 1999; Easton et al., 2005]. Murthy et al. [2000] mapped the *AKT3* gene to chromosome 1q44 by FISH study. *AKT3* represents an excellent candidate for developmental human microcephaly and ACC. It has been suggested that haploinsufficiency causes postnatal microcephaly and ACC [Boland et al., 2007; Andrieux et al., 2008].

In the literature, *AKT3* has been reported to be associated with corpus callosal abnormality. De Vries et al. [2001] reported 2 unrelated mentally retarded boys with a clinical pattern of growth retardation (prenatal onset), severe progressive microcephaly, hypospadias, corpus callosum abnormalities, cardiac anomalies, and gastro-oesophageal reflux. One showed a submicroscopic distal 1q deletion, and the other showed a partial submicroscopic trisomy of distal 13q in addition to a submicroscopic distal 1q deletion. Gentile et al. [2003] described a case with minor facial anomalies, mental retardation, seizures, and partial ACC with de novo chromosome 1q43q44 deletion. Boland et al. [2007] defined a 3.5-Mb critical region in 1q44 containing one or more genes leading to microcephaly and corpus callosum abnormalities when present in only one functional copy. Mapping of a balanced reciprocal t(1;13)(q44;q32) translocation in a patient with postnatal microcephaly and ACC demonstrated a breakpoint in this region that was situated 20 kb upstream of *AKT3*. The murine ortholog *AKT3* is required for the developmental regulation of normal brain size and callosal development. Whole-mount in situ hybridization confirmed expression of *AKT3* in the developing CNS during mouse embryogenesis.

Hill et al. [2007] described a case of distal deletions of chromosome 1q with microcephaly, hypoplasia or ACC, and psychomotor retardation. Using microsatellite and SNP markers, they mapped the deleted regions in 7 patients with terminal deletions to 1q431q44. Andrieux et al. [2008] reported a boy with developmental delay, growth retardation, facial dysmorphisms, vermis hypoplasia, micropolygyria, ACC and a normal karyotype. High-resolution oligonucleotide array CGH showed a de novo 6.9-Mb 1qter deletion/4.4-Mb 18pter duplication. van Bon et al. [2008] described the clinical presentation of 13 new patients with a submicroscopic deletion of 1q43q44. The clinical presentation of these patients had

clear similarities with previously reported cases with a terminal 1q deletion. Corpus callosum abnormality was present in 10 of these patients. Orellana et al. [2009] reported one patient with the clinical and molecular findings consistent with a role for *AKT3* in corpus callosum development. Shimojima et al. [2012] also described 6 patients with ACC and 1q44 deletion including *AKT3*.

Boland et al. [2007] sequenced *AKT3* in 45 patients and no mutations were found. Poot et al. [2007] reported a patient with vermis hypoplasia, dilatation of the fourth ventricle, enlarged cisterna magna, and ACC. They identified a 5-Mb microscopic terminal deletion of 1q44, but no deletion of *AKT3*. van Bon et al. [2008] sequenced the *AKT3* gene in 19 patients with ACC, but did not detect any abnormalities. They also found *AKT3* deletions in 2 sisters with ACC and their unaffected mother. However, they were able to delineate the critical region for corpus callosum abnormalities to a 360-kb genomic segment which contains 4 possible candidate genes, but excluding the *AKT3* gene. Furthermore, a normal copy number of *AKT3* with a more distal 1q44 deletion can still lead to corpus callosum abnormalities. Caliebe et al. [2010] described 2 patients with speech delay, seizures and variable corpus callosum thickness and overlapping deletions in the chromosomal region 1q44, but there was no deletion of *AKT3*. ACC appears to be a landmark phenotype for deletion 1q44 syndrome, and the critical genes are proximal to *SMYD3* in the 1q44 region, excluding the *AKT3* gene [Lall et al., 2011].

Some of the recent literature point towards other genes to be associated with corpus callosum abnormality. Ballif et al. [2012] studied 22 patients with deletion of 1q43q44 and proposed *ZNF238* as a candidate gene for ACC, which was also suggested by Perlman et al. [2013]. Nagamani et al. [2012] studied 7 patients with deletions of chromosome 1q43q44 and ruled out the involvement of *AKT3* and implicated *CEP170*, and/or *ZNF238* as novel genes causative for ACC in patients with a terminal 1q deletion. *CEP170* is expressed extensively in the brain and encodes for a protein that is a component of the centrosomal complex. Caliebe et al. [2010] and Selmer et al. [2012] suggested the *HNRPU* gene, involved in the regulation of embryonic brain development, as the candidate gene for ACC in patients with 1q44 deletion. Exome sequencing studies identified homozygous and compound heterozygous mutations in *C12orf57* to cause an autosomal recessive syndromic form of intellectual disability with ACC. *C12orf57* is a highly conserved gene and is required for the development of the human corpus callosum [Akizu et al., 2013; Platzer et al., 2014].

Conclusion

In this study, we did not identify an *AKT3* deletion in any of the patients; however, we have not ruled out the point mutation in *AKT3*. Therefore, a larger cohort of samples is needed to establish the association of the *AKT3* gene with ACC. Further research is necessary in understanding the genetics and pathogenesis of ACC and its effect in the fetus.

Acknowledgment

This study was supported in part by the CAS Project expenses for clinical research from IBM India Pvt. Ltd.

Statement of Ethics

The study was approved by the Institutional Ethics Committee. Informed consent was obtained from each family.

Disclosure Statement

The authors have no conflicts of interest to declare.

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