ARTICLE

Delineation of a deletion region critical for corpus callosal abnormalities in chromosome 1q43–q44

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Submicroscopic deletions involving chromosome 1q43–q44 result in cognitive impairment, microcephaly, growth restriction, dysmorphic features, and variable involvement of other organ systems. A consistently observed feature in patients with this deletion are the corpus callosal abnormalities (CCAs), ranging from thinning and hypoplasia to complete agenesis. Previous studies attempting to delineate the critical region for CCAs have yielded inconsistent results. We conducted a detailed clinical and molecular characterization of seven patients with deletions of chromosome 1q43–q44. Using array comparative genomic hybridization, we mapped the size, extent, and genomic content of these deletions. Four patients had CCAs, and shared the smallest region of overlap that contains only three protein coding genes, *CEP170*, *SDCCAG8*, and *ZNF238*. One patient with a small deletion involving *SDCCAG8* and *AKT3*, and another patient with an intragenic deletion of *AKT3* did not have any CCA, implying that the loss of these two genes is unlikely to be the cause of CCA. *CEP170* is expressed extensively in the brain, and encodes for a protein that is a component of the centrosomal complex. *ZNF238* is involved in control of neuronal progenitor cells and survival of cortical neurons. Our results rule out the involvement of *AKT3*, and implicate *CEP170* and/or *ZNF238* as novel genes causative for CCA in patients with a terminal 1q deletion.

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INTRODUCTION

Submicroscopic deletions involving the distal region of chromosome 1q result in a complex phenotype that includes growth restriction, microcephaly, craniofacial dysmorphisms, significant cognitive impairment, hypotonia, and corpus callosal abnormalities (CCAs).^{1–6} CCAs, ranging from thinning and hypoplasia to complete agenesis, are consistently observed in deletions involving terminal 1q.^{1–6} Previous studies have suggested that the deletion of the most telomeric part of the long arm of chromosome 1, 1q43–q44, is responsible for the CCAs. However, narrowing the critical region responsible for CCAs has yielded inconsistent results.^{1,6–9} Boland *et al*⁸ mapped the deletion and translocation breakpoints in 1q44, and suggested that haploinsufficiency of *AKT3* is responsible for the CCAs (Figure 1). However, Poot *et al*^{9,10} described CCAs in a patient with terminal deletion of 1q44, without involvement of *AKT3*. A subsequent study by van Bon *et al*¹ narrowed the critical region for CCAs containing only *C1orf100*, *ADSS*, *C1orf101*, and *PNAS-4*. A more recent study hypothesized the gene *HNRPU* that is telomeric to the critical region mapped by van Bon *et al*¹, is causative of CCAs (Figure 1).⁶

Using a reverse genomic approach, we sought to better understand the genotype – phenotype correlations, and map the critical region for CCAs in patients with deletion of 1q43–q44.¹¹ The Medical Genetics

Laboratories at Baylor College of Medicine (BCM) has performed over 14 000 array comparative genomic hybridization (aCGH) for clinical evaluation of subjects with developmental delay, dysmorphic features, and/or multiple congenital anomalies from June 2007 to January 2011. From this cohort of patients, we identified seven patients with deletions of 1q43–q44 including one patient with an intragenic deletion of *AKT3*. In this report, we describe the delineation of a critical region for CCAs and show that deletion of a region spanning ~930 kb between genomic coordinates 241 054 360–241 522 998 and 241 936 310–242 398 193 (NCBI36/hg18) is responsible for the CCAs (Figure 1).

METHODS

Human subjects

Identification of 1q43–q44 deletions was made by clinical diagnostic testing using aCGH. Clinical information was obtained from health care providers, using a checklist to standardize the data collection. The protocol was approved by the Institutional Review Board for human subjects' research at BCM.

Array comparative genomic hybridization

We performed aCGH analysis on the clinical microarray platform routinely used in our institution. The microarrays were designed in the Medical Genetics

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Figure 1 Size, extent, and genomic content of deletions of 1q43–q44, and mapping of critical region for CCAs and microcephaly. Upper panel depicts the ideogram of chromosome 1 with the genomic coordinates and the gene content of the 1q43–q44 region. The red bars depict the deletions in our cohort of patients, with blue stars adjacent to the bars representing the patients with CCAs, and the green triangles depicting the patients with microcephaly. The blue colored genomic interval that encompasses *CEP170* and *ZNF238* is the critical region for CCAs, whereas the green interval depicts that loss of *AKT3* is critical for microcephaly. The previously mapped regions for CCA by Boland *et al*⁸, van Bon *et al*¹ and Caliebe *et al*⁶ are depicted at the bottom. The deletions in patients 1–3 were confirmed to be *de novo*, the deletion in patient 7 was inherited from a clinically asymptomatic mother, whereas the inheritance pattern in the remaining three patients could not be ascertained.

Laboratory of BCM. All of the patient samples were interrogated using V8.OLIGO (180K). The V8.OLIGO is a custom-designed array with approximately 180 000 (60 mer) interrogating oligonucleotides, manufactured by Agilent Technologies, Inc. (Santa Clara, CA, USA). This array contains the 'best-performing' oligonucleotides (oligos) selected from Agilent's online library (eArray; <https://earray.chem.agilent.com/earray/>) and has been further optimized using empiric data. This array is designed to provide interrogation of all known microdeletion and microduplication syndrome regions, as well as pericentromeric and subtelomeric regions as previously described.¹² In addition, 1784 genes either known to cause or hypothesized as candidate genes for various clinical phenotypes have exonic coverage with an average of 4.2 probes per exon, as well as introns greater than 10 kb. The entire genome is covered with an average resolution of 30 kb, excluding low-copy repeats and other repetitive sequences (<https://www.bcm.edu/geneticlabs/>). The procedures for DNA digestion, labeling, hybridization, and data analysis, were performed as previously described.¹³

RESULTS

Array comparative genomic hybridization

The deletions ranged in size from 0.08 to 4.35 Mb (Table 1). The deletions in patients 1–3 were confirmed to be *de novo*, the deletion in patient 7 was inherited from a clinically asymptomatic mother, whereas the inheritance pattern in the remaining three patients could not be ascertained due to non-availability of both parental samples. All deletions involved at least a portion of the *AKT3* gene, except for patient 7. Two deletions were intragenic; the first disrupted *AKT3* gene in patient 5, and the second disrupted *SMYD3* gene in patient 7 (Figure 1). All deletions were confirmed by FISH analysis (data not shown). We did not detect any other copy number variations that could confound the interpretation of the data.

Clinical features

The clinical features of patients are summarized in Table 2. As the mother of patient 7 did not have phenotypic consequences, patient 7 was

excluded from analysis. Patients with deletions of 1q43–q44 predominantly presented with a neuro-cognitive phenotype with developmental delay involving gross motor, fine motor, and language faculties. Microcephaly and/or structural abnormalities of the brain were observed in all, except patient 6. CCAs ranging from severe thinning to complete agenesis were seen in patients 1 through 4, whereas patients 5 and 6 with small deletions did not have any CCAs. Congenital heart disease in the form of atrial septal defect, Tetralogy of Fallot, and ventricular septal defect were noted in patients 3, 4, and 5, respectively. Craniofacial dysmorphic features were generally mild, with no characteristic facial gestalt.

DISCUSSION

Submicroscopic deletions involving the terminal portion of chromosome 1q43–q44 have recently emerged as a recognizable phenotype. The deletions in this region are the most common CNV associated with CCAs, and 35 patients have been reported to date.¹⁴ Previous studies attempting to map the critical region for CCAs observed with the deletions of this region have yielded inconsistent results. Boland *et al*⁸ studied patients with unbalanced structural rearrangements of distal 1q, and narrowed down a 3.5-Mb critical region that they hypothesized to contain the gene responsible for CCA. By further mapping of a t(1;13)(q44;q32) in a patient with microcephaly and CCA, they demonstrated a breakpoint mapping 20 kb upstream of *AKT3*, a serine–threonine kinase, and suggested that haploinsufficiency of this gene was responsible for CCA. Orellana *et al*⁷ described a patient with a *de novo* small interstitial 1q44 deletion mapping from *ADSS* to *KIF26B*, with a mild phenotype and absence of CCA consistent with a critical region encompassing *AKT3* or *ZNF238* genes in corpus callosum development. Two targeted *Akt3* knockout mouse models have been described,^{15,16} and one demonstrated callosal hypoplasia¹⁶ with loss of both copies of *Akt3*. These data suggested that deletion of *AKT3* is responsible for CCAs.

Table 1 Molecular mapping of deletions in chromosome 1q43–q44

Patient	Centromeric breakpoint	Telomeric breakpoint	Deletion size (Mb)	Genes encompassed by the deletion	Inheritance
1	240 640 553–240 683 463	243 824 577–243 882 928	3.15	<i>PLD5</i> → <i>KIF26B</i>	<i>de novo</i>
2	241 014 694–241 054 360	242 323 495–242 398 193	1.26	<i>LOC731257</i> → <i>ZNF238</i>	<i>de novo</i>
3	241 014 694–241 054 360	244 584 985–244 616 824	4.35	<i>MAP1LC3C</i> → <i>SYMD3</i>	<i>de novo</i>
4	240 228 748–241 119 642	245 037 854–245 059 290	3.93	<i>LOC731257</i> → <i>LOC149134</i>	Mother does not have CNV, father unavailable for testing
5	241 833 595–241 843 404	241 925 842–241 936 310	0.08	<i>AKT3</i>	Unknown
6	241 505 679–241 522 998	241 735 307–241 742 028	0.23	<i>SDCCAG8</i> → <i>AKT3</i>	Mother does not have CNV, father unavailable for testing
7	244 240 651–244 279 769	244 744 924–244 757 295	0.517	<i>SYMD3</i>	Maternal

The data represent the location of breakpoints and the size of the deletions as assessed by V8.OLIGO.

Table 2 Clinical features of patients with deletion of 1q43–q44

Patient	1	2	3	4	5	6
Age (years)	2	7	6	11	3.5	17
Sex	M	F	F	M	F	F
Age at evaluation	6 months	2 years	3 years	9 years	11 months	7.5 years
Reason for aCGH	Right sex cord stromal tumor, GDD	GDD, microcephaly, CCA	Microcephaly	Microcephaly, GDD, seizures, TOF	Microcephaly, genito-urinary abnormalities, VSD	Mild DD
FOC (centile)	<3	<5	<5	<3	<3	50
Wt (centile)	40	<5	<5	30	25	57
HT (centile)	90	50	<5	NA	50	75
Cranio-facial dysmorphism						
Head	Microcephaly, flat occiput	Microcephaly	Microcephaly with brachycephaly	Microcephaly	Microcephaly	Normal
Facial dysmorphic features	—	Hypertelorism	Depressed nasal bridge	—	+	—
Nervous system						
Developmental delay	GDD	GDD	GDD	GDD	GDD	Mild DD
Seizures	+	—	—	+	—	—
Hypotonia	+	—	—	—	—	—
Behavioral issues	—	Aggressive	—	—	—	Anxiety
Structural brain abnormalities	ACC, temporal pachygyria, occipital polymicrogyria	Thinning of corpus callosum and hypoplasia of splenium, prominent cisterns with cerebral volume loss	ACC, mild simplification of cerebral gyri	Severe thinning of corpus callosum, small cerebral hemispheres, dilated subarchnoid space, cerebellar atrophy	None	None
Heart	None	None	ASD	TOF	VSD	None
Other organ system involvement	Sex cord stromal tumor	None	Polysyndactyly of toes	Hirschsprung disease	Renal hypoplasia and ureteral duplication	None

Abbreviations: ACC, agenesis of corpus callosum; CCA, corpus callosum abnormalities; DD, developmental delay; GDD, global developmental delay; NA, data not available, TOF, Tetralogy of Fallot; VSD, ventricular septal defect.

However, in a case report by Poot *et al*,¹⁰ a patient with a terminal 1q44 deletion that spared both *ZNF238* and *AKT3* did have CCA, implying that *AKT3* may not be causative of this abnormality. van Bon *et al*¹ in their cohort of 13 patients with the submicroscopic 1q43–q44 deletions delineated a region that was telomeric to and excluding *AKT3*, mapping from *C1orf100* to *PNAS-4* for CCAs. Caliebe *et al*⁶ showed that their cohort of four patients with

a terminal 1q deletion and CCA shared a 0.44-Mb region of overlap, containing the genes *FAM36A*, *HNRPU*, *EFCAB2*, and *KIF26B*, telomeric to the one mapped by van Bon *et al* (Figure 1). Although these studies implied that *AKT3* maps outside of the region(s) critical for CCA, they do not address the question as to whether these deletions telomeric to *AKT3* affect the transcription of *AKT3*.

Our study reports an intragenic deletion of *AKT3* in a patient with no evidence of CCAs, conclusively showing that haploinsufficiency of this gene is not responsible for this abnormality. The deletion in patient 2 is the smallest one amongst our patients with CCA. As patients 5 and 6 do not have any CCA, the region between genomic coordinates 241 054 360 and 241 522 998, centromeric to *AKT3* encompassing *CEP170*, and the region between 241 936 310 and 242 398 193 containing *ZNF238* is likely critical for development of CCA (Figure 1). *CEP170* is expressed extensively in the brain and encodes for a protein that is a component of the centrosomal complex that is involved in maintaining microtubule organization and function. Centrosomal proteins have a well-known function in neurogenesis, and have been hypothesized as important genes for the evolution of human brain size. Mutations in centrosomal proteins cause microcephaly.^{17,18} Recently, it has been demonstrated that centrosomal proteins are essential for microtubule organization and centrosome motility that have important roles in axonal formation.¹⁹ *ZNF238* is a POZ/zinc finger transcriptional repressor gene.²⁰ Mice with targeted knock out of *ZNF238* display severe hypoplasia of the cerebral cortex and the hippocampus, along with increased apoptosis of progenitors in the ventricular zone.²¹ These functional data and the genotype–phenotype correlation in our patients implicate deletion of *CEP170* and/or *ZNF238* as the cause for CCAs. Although our smallest region of overlap is consistent with the critical regions for CCAs previously mapped by Boland *et al*⁸ and Orellana *et al*,⁷ it is centromeric to those mapped by other studies,^{1,6,10} implying that the 1q43–q44 region harbors more than one gene important for the development of a normal corpus callosum.

In addition, we describe the first intragenic deletion of *AKT3* and the associated phenotypic consequences that include renal abnormalities, a septal heart defect, microcephaly, and developmental delay in the absence of CCA. All patients with deletion of *AKT3* had microcephaly, except patient 6. The CNV in patient 6 deletes only the last exon of *AKT3*, and hence, may lead to residual protein being translated. Although we could not confirm whether the intragenic deletion was *de novo*, all patients with deletion of *AKT3* reported to date have been observed to have microcephaly. The only report of deletion of *AKT3* without any phenotypic manifestations is that of an unaffected mother of two children with microcephaly and developmental delay described by van Bon *et al*.¹ In the same report, the only patient without microcephaly had a deletion that spared *AKT3*. Both of the existing knock out mouse models of *Akt3* deficiency showed postnatal microcephaly.^{15,16} Our data interpreted, along with existing functional data, suggests that *AKT3* is indeed important for the normal development of the brain, but not for CCA.

In summary, deletions of 1q43–q44 result in microcephaly, developmental delay, and corpus callosal abnormalities. *AKT3* is important for normal brain development, and its haploinsufficiency causes microcephaly, but not corpus callosal abnormalities. Deletion of the novel centrosomal protein *CEP170* and/or the zinc finger transcriptional repressor *ZNF238* causes corpus callosal abnormalities.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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