Corpus callosum abnormalities, intellectual disability, speech impairment, and autism in patients with haploinsufficiency of ARID1B

Corpus callosum abnormalities are common brain malformations with a wide clinical spectrum ranging from severe intellectual disability to normal cognitive function. The etiology is expected to be genetic in as much as 30–50% of the cases, but the underlying genetic cause remains unknown in the majority of cases. By next-generation mate-pair sequencing we mapped the chromosomal breakpoints of a patient with a de novo balanced translocation, t(1;6)(p31;q25), agenesis of corpus callosum (CC), intellectual disability, severe speech impairment, and autism. The chromosome 6 breakpoint truncated ARID1B which was also truncated in a recently published translocation patient with a similar phenotype. Quantitative polymerase chain reaction (Q-PCR) data showed that a primer set proximal to the translocation showed increased expression of ARID1B, whereas primer sets spanning or distal to the translocation showed decreased expression in the patient relative to a non-related control set. Phenotype–genotype comparison of the translocation patient to seven unpublished patients with various sized deletions encompassing ARID1B confirms that haploinsufficiency of ARID1B is associated with CC abnormalities, intellectual disability, severe speech impairment, and autism. Our findings emphasize that ARID1B is important in human brain development and function in general, and in the development of CC and in speech development in particular.

Conflict of interest

The authors declare no conflict of interests.

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The corpus callosum (CC) is the main interhemispheric commissure transferring cognitive, sensory, and motor information between the two brain hemispheres. CC abnormalities include complete agenesis, hypoplasia, and varied degrees of partial agenesis (1). Agenesis of CC (ACC) occurred in 1 in 1000 in a series of unselected neonates (2) and is thus one of the most common brain malformations. It is a heterogeneous condition with a wide clinical spectrum ranging from severe intellectual disability to normal cognitive function (3, 4). The etiology is believed to be genetic in 30–50% of the cases (5, 6) whereas fetal infections and exposure to teratogens, e.g. alcohol, are suspected causes in the remaining cases. Numerous chromosomal loci have been associated with ACC (7, 8) including loci at 6q25-q27 (8–11), but the underlying genetic cause remains unknown in the majority of cases.

Here we report eight previously unpublished patients with haploinsufficiency of ARID1B: one patient with a de novo translocation t(1;6)(p31;q25) mapped by next-generation sequencing (NGS) and seven patients with various sized de novo deletions.

Materials and methods

Patients

Each patient was clinically and molecularly evaluated by at least one of the authors. Patient 1 was identified through a national study of carriers of structural rearrangements; the study was approved by the Danish Scientific Ethics Committee and the Danish Data Protection Agency and written informed consent was obtained. Patients 2–8 were referred to genetic evaluation due to developmental delay; informed consent was obtained at the local clinical genetics departments. Patients 3–8 were identified in DECIPHER (12).

Chromosome analysis

Standard G-banding chromosome analysis was performed on cultured peripheral lymphocytes.

Next-generation paired-end sequencing

Mate-pair libraries were prepared using the Mate Pair Library v2 kit (Illumina, San Diego, CA). Briefly, 10 μg genomic DNA was sheared using a Nebulizer. Fragments of 2–3 kb were isolated, end-repaired using a mix of natural and biotinylated dNTPs, blunt-end ligated using circularization ligase, and fragmented to 200–400 bp. Biotinylated fragments were isolated and end-repaired and A-overhangs were added to the 3’ ends. Paired-end adapters were ligated to the fragments and the library was amplified by 18 cycles of PCR. Mate-pair libraries were subjected to 2 × 36 bases paired-end sequencing on a Genome Analyzer IIx (Illumina), following the manufacturers protocol. Reads were aligned to a reference genome using Bowtie (13) allowing up to two mismatches in the seed region. Reads not aligning uniquely were discarded from further analysis. Paired reads aligning to different chromosomes or with unexpected strand orientation were extracted to identify potential translocation and inversion breakpoints, respectively. Breakpoints were only considered as candidates if they were confirmed by at least three independent paired reads with end-reads mapping within a 6 kb region. Predicted breakpoints were filtered against known in-house variants based on data from 30 individuals with known breakpoints. Breakpoints were confirmed by PCR amplification and Sanger sequencing of the breakpoint-spanning fragments.

Quantitative polymerase chain reaction

RNA from patient 1 and five controls was extracted from peripheral blood using standard procedures. Following extraction, RNA was DNase I (Invitrogen, San Diego, CA) treated and reverse transcribed with a HT11V primer using SuperscriptII (Invitrogen). Primers for ARID1B were designed using oligo software (Molecular Biology Insights Inc., W. Cascade, CO) (Table S1, Supporting information). All primer sets were designed to span at least one intron. Q-PCR was performed on an Opticon3 thermocycler (Bio-Rad Laboratories, Hercules, CA). All samples were run in triplicates. Normalization of expression was done using two stable housekeeping genes (EIF6 and G6PD). Assessment of stable housekeeping genes was done using GENORM software (14).

Microarray analysis

Patient 1 was examined with Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA). Copy number variations (CNVs) > 1 kb and detected by at least eight markers were identified using the GENOTYPING CONSOLE software (Affymetrix) and compared with variants reported in the Database of Genomic Variants.
Patient 2 was examined with Agilent Oligoarray 400K, patient 3 with Affymetrix 250K SNP array, patients 4 and 8 with Agilent 44K, patient 5 with Affymetrix 250K and Illumina Sentrix HumanHap300, patient 6 with Agilent Human Genome CGH Microarray 44 B, and patient 7 with Agilent Oligoarray 244K.

Results

All position coordinates given below are based on Human Feb. 2009 (GRCh37/19) assembly.

Clinical reports

Clinical data are provided in Table 1. Full clinical reports are provided in Appendix S1, Supporting information.

Patient 1

Patient 1 is an 8-year-old male. He was the first child of healthy unrelated parents. Routine second trimester ultrasound examination showed enlarged cerebral ventricles; amniocentesis was performed and a de novo balanced reciprocal translocation t(1;6)(p31;q25) was detected. The patient was born at term with a birth weight of 2450 g, birth length of 48 cm, and occipital frontal circumference (OFC) of 33 cm. He was hypotonic and had mild dysmorphic features. Developmental milestones were significantly delayed; he sat at the age of 2, walked at 2 1/2, and spoke one to two words at the age of 3. Feeding problems and severe constipation were prominent from 6 months until 3 years of age. At the latest clinical examination, 8 years old, height was 120 cm, weight 21.8 kg, and OFC 54 cm. Dysmorphic features included a small triangular face, low hairline, micrognathia, small pointed chin, low-set large ears, broad nasal bridge and tip, concave curved thin vermilion of the upper lip, camptodactyly, and deep longitudinal plantar creases between first and second toes. He spoke only few words. Magnetic resonance imaging (MRI) showed complete ACC, and he was diagnosed with intellectual disability and autism according to the Global Assessment of Psychosocial Disability (GAPD) with intellectual disability and autism according to the

Chromosome analysis

Chromosome analysis confirmed the karyotype 46,XY, t(1;6)(p31;q25) in patient 1 (Fig. 1a).
Brain malformations in patients with ARID1B haploinsufficiency

Table 1. Clinical and molecular characterization of patients with ARID1B haploinsufficiency

|                  | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 | Patient 8 |
|------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Gender           | M         | F         | M         | F         | F         | F         | F         | F         |
| Age (years)      | 9         | 3         | 46        | 8         | 4         | 20        | 9         | 10        |
| Birth (gestational week) | 39       | 40        | —         | 39        | 39        | 40        | 41        | 39        |
| Birth length (cm) | 48       | 49        | —         | 44.5      | —         | 50        | 46        | 45.5      |
| Birth weight (g) | 2450      | 3090      | —         | 2390      | 2770      | 3050      | 2890      | 2640      |
| OFC at birth (cm)| 33        | —         | —         | 32        | —         | 33        | 33.5      | 32.5      |
| **Clinical findings** | | | | | | | | |
| ID/DD            | Yes       | Yes       | Yes       | Yes       | Yes       | Yes       | Yes       | Yes       |
| ACC              | Yes       | Not examined | Not examined | No        | Not examined | Partial | Partial | Hypoplasia |
| ASD              | Yes       | Autistic traits | Autistic traits | Yes       | —         | Yes       | No        | No        |
| Severe speech impairment | Yes | Yes | Absent speech | Yes | Absent speech | Absent speech | Yes | Yes |
| Seizures         | No        | No        | —         | No        | No        | Yes       | Yes       | Yes       |
| Hypotonia        | Yes       | Yes       | —         | Yes       | Yes       | Yes       | Yes       | Yes       |
| Low hairline     | Yes       | —         | —         | Yes       | —         | Yes       | Yes       | Yes       |
| Low-set ears     | Yes       | —         | —         | —         | —         | —         | —         | Yes       |
| Broad nasal tip  | Yes       | —         | —         | —         | —         | Yes       | —         | Yes       |
| Thin vermilion of upper lip | Yes | No | — | Yes | — | Yes | — | — |
| Hypertrichosis   | No        | Yes       | —         | —         | —         | —         | Yes       | Yes       |
| Pectus excavatum | No        | Yes       | —         | —         | —         | —         | Yes       | Yes       |
| Joint laxity     | No        | Yes       | Yes       | —         | —         | —         | Yes       | Yes       |
| Vision           | Hypermetropia | —       | Myopia    | Strabismus | —         | Myopia, strabismus | Hypermetropia, nystagmus | Cataracts |
| **Growth**       | | | | | | | | |
| Feeding problems in infancy/failure to thrive | Yes | Yes | — | — | — | Yes | Yes | Yes |
| Height (age)     | 117 cm (−1.8 SD) (7.5 years) | 84 cm (−2.5 SD) (2 years) | 1.59 m (−3.5 SD) (adult) | — | 66.8 cm (−2 SD) (9 months) | 152 cm (fifth centile) (18 years) | 124 cm (−2 SD) (8 years 9 months) | 112 cm (−4 SD) (9.5 years) |
| Weight (age)     | 20 kg (−1.6 SD) (7.5 years) | 12 kg (−1.2 SD) (2 years) | 71 kg (adult) | — | 5.6 kg (−2 SD) (9 months) | 48 kg (10–25th centile) (18 years) | 26.5 kg (−0.5 SD) (8 years 9 months) | 19.5 kg (−2 SD) (9.5 years) |
| OFC (age)        | 54 cm (−1.4 SD) (7.5 years) | 42.5 (−0.7 SD) (8 months) | 56.5 cm (−0.75 SD) (adult) | — | 43.9 cm (0 SD) (9 months) | 54 cm (25–50th centile) (18 years) | — | 48.5 cm (−2.5 SD) (9.5 years) |
| Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 | Patient 8 |
|----------|----------|----------|----------|----------|----------|----------|----------|
| **Molecular characterization** | | | | | | | |
| Karyotype | 46,XY,t(1;6) (p31.1;q25.3)dn | 46,XX | 46,XY | 46,XX | 46,XX | 46,XX | 46,XX |
| Microarray platform | Affymetrix SNP 6.0 | Agilent Oligoarray 400K | Affymetrix 250K SNP array | Agilent 44K | Affymetrix 250K; Illumina Sentrix HumanHap300 | Agilent Human Genome CGH Microarray 44B | Agilent Oligoarray 244K |

| Deletion (size)b | No pathogenic deletions | 0.2 Mb | 0.6 Mb | 1.0 Mb | 2.7 Mb | 4.6 Mb | 8.2 Mb | 14.5 Mb |

| Minimal deleted region on chromosome 6 (genomic position, hg19)c | | | | | | | |
| ARID1B, intron 5 | ARID1B, exons 4–8 | ARID1B, exons 2–20 | ARID1B, exons 1–8 | 5 RefSeq genes incl. ARID1B | 15 RefSeq genes incl. ARID1B, exons 1–7 | 33 RefSeq genes incl. ARID1B | 73 RefSeq genes incl. ARID1B |

ACC, Agenesis of corpus callosum; ASD, autism spectrum disorder; DD, developmental delay; F, female; ID, intellectual disability; M, male; OFC, occipital frontal circumference; SD, standard deviation.

aWhen no information was available regarding a specific clinical feature the item was not scored.
bAll deletions were de novo.
cTheoretical maximal deleted region is given in brackets.
dARID1B RefSeq transcript NM_020732.3.
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Fig. 1. Cytogenetic and molecular characterization of patient 1. (a) Partial karyotype, showing a de novo balanced reciprocal translocation involving chromosomes 1 and 6. Cytogenetic karyotype: 46,XY,t(1;6)(p31;q25)dn. Blue arrows indicate the cytogenetically determined breakpoints. (b) The translocation breakpoints were mapped using next-generation mate-pair sequencing. The chromosome 1 breakpoint mapped within a 3 kb non-genic region at 1p31.1 (shaded area) and the chromosome 6 breakpoint mapped within a 700 bp genomic region at 6q25.3 (shaded area), truncating ARID1B. The breakpoints were detected by three reads shown in green and blue (the colors indicate the strand orientation of the reads). (c) By Sanger sequencing, the exact genomic positions of the breakpoints were identified to chr1:73,895,566-73,895,579 and chr6:157,292,076-157,292,079 (hg19). Four base pairs (TAGA) of unknown origin were inserted at the (der)(1) breakpoint (shaded area) and 23 base pairs (TCTGCAGAAAGTATAGGTCTGAT) were inserted at the der(6) breakpoint (shaded area); 22 of these (TCTGCAGAAAGTATAGGTCTGA) match uniquely to a LINE sequence on chromosome 7.

Fig. 2. Expression pattern for ARID1B in patient 1 compared to five controls. Expression levels in patient 1 using primers downstream of the translocation site were roughly half of that in the five controls. Expression levels in patient 1 using a primer set spanning the translocation likewise showed that expression was halved. Expression data obtained with a primer set located upstream of the translocation showed that the expression in patient 1 was roughly twice that of the average of the controls.

likely to impact the observed phenotypes. Brain MRI was not performed on three of the reported patients; as this procedure would require general anesthesia it was decided against for ethical reasons. Despite these obvious limitations, overlapping clinical manifestations were present: all eight patients had intellectual disability, severe speech impairment, and various degrees of dysmorphic features. Callosal abnormalities were present in four of the five patients where brain imaging was performed. Three patients were diagnosed with ASD and another two showed autistic traits. This is in accordance with two recently published reports describing (i) a small de novo deletion within ARID1B in a patient with autism (15) and (ii) a patient with ACC, intellectual disability, speech impairment, and autism, in which a de novo translocation disrupted two genes: ARID1B and MRPP3 (16). Patient 4 had normal brain MRI; this is not surprising as ACC associated loci are known to exhibit reduced penetrance (8, 11, 17). Interestingly, that same patient had intellectual disability, speech impairment, and ASD, suggesting that these traits might not be associated with visible structural brain abnormalities.
As ARID1B is disrupted by the translocation in patient 1, the expression of the gene could be expected to be halved unless compensatory expression was done from the normal chromosome. The observed expression pattern for primer sets downstream of or spanning the translocation was in concordance with these expectations. As data sets for exons 5–7 and 7–8 exhibit virtually identical relative expression levels, it can be indirectly inferred that the downstream fragment carrying ARID1B, translocated onto the derivative chromosome 1, is transcriptionally inactive as would be expected because this fragment carries no promoter region. The expressional pattern of exons located upstream of the translocation (exons 4–5) indicates that ARID1B is expressed at levels higher than for amplicons downstream of the translocation. This indicates that ARID1B is not only transcriptionally active on the normal chromosome but also from the fragment on der(1)
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Supporting Information

The following Supporting information is available for this article: Table S1. Primers used for Q-PCR examination of expression levels of ARID1B in patient 1. Appendix S1. Clinical reports of patients 2–8. Additional Supporting information may be found in the online version of this article. Please note: Wiley-Blackwell Publishing is not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

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