ORIGINAL ARTICLE

# Eight further individuals with intellectual disability and epilepsy carrying bi-allelic *CNTNAP2* aberrations allow delineation of the mutational and phenotypic spectrum

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## ABSTRACT

**Background** Heterozygous copy number variants (CNVs) or sequence variants in the contactin-associated protein 2 gene *CNTNAP2* have been discussed as risk factors for a wide spectrum of neurodevelopmental and neuropsychiatric disorders. Bi-allelic aberrations in this gene are causative for an autosomal-recessive disorder with epilepsy, severe intellectual disability (ID) and cortical dysplasia (CDFES). As the number of reported individuals is still limited, we aimed at a further characterisation of the full mutational and clinical spectrum.

**Methods** Targeted sequencing, chromosomal microarray analysis or multigene panel sequencing was performed in individuals with severe ID and epilepsy.

**Results** We identified homozygous mutations, compound heterozygous CNVs or CNVs and mutations in *CNTNAP2* in eight individuals from six unrelated families. All aberrations were inherited from healthy, heterozygous parents and are predicted to be deleterious for protein function. Epilepsy occurred in all affected individuals with onset in the first 3.5 years of life. Further common aspects were ID (severe in 6/8), regression of speech development (5/8) and behavioural anomalies (7/8). Interestingly, cognitive impairment in one of two affected brothers was, in comparison, relatively mild with good speech and simple writing abilities. Cortical dysplasia that was previously reported in CDFES was not present in MRIs of six individuals and only suspected in one.

**Conclusions** By identifying novel homozygous or compound heterozygous, deleterious CNVs and mutations in eight individuals from six unrelated families with moderate-to-severe ID, early onset epilepsy and behavioural anomalies, we considerably broaden the mutational and clinical spectrum associated with bi-allelic aberrations in *CNTNAP2*.

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## INTRODUCTION

*CNTNAP2* is one of the largest genes in the human genome, consisting of 24 exons (NM_014141) and spanning 2.3 Mb on chromosome 7q35-36.1.[1] It encodes contactin-associated protein 2 (CASPR2), a transmembrane protein which is distantly related to the family of neurexins and regulates neuron-glia contact in vertebrates and glia-glia contact in insects.[2] The large extracellular region of CASPR2 contains several domains, including four laminin G domains, two epidermal growth factor-like domains and discoidin/neuropilin and fibrinogen-like domains. The small C-terminal cytoplasmatic part contains a PDZ domain-binding sequence that is supposed to mediate interactions with PDZ-containing proteins.[3 4] *CNTNAP2* is highly expressed in the spinal cord and several brain regions, particularly in a cortico-striato-thalamic circuit that is involved in diverse higher-order cognitive functions.[5 6] Vertebrate Caspr2 colocalises with potassium channels in the juxtaparanodal regions of Ranvier nodes in myelinated axons.[4 7] It has roles in brain development and function by being involved in processes such as neuronal migration, dendritic arborisation and spine development.[8–10]

Heterozygous chromosomal aberrations, copy number variants (CNVs) and sequence variants in *CNTNAP2* have been implicated as incompletely penetrant risk factors in a wide spectrum of neurodevelopmental and neuropsychiatric disorders. These include Tourette syndrome, intellectual disability (ID), autism-spectrum disorders, speech and language impairment, epilepsy and schizophrenia.[6 11–18] The presence of one or more additional contributing risk factors in symptomatic carriers is likely.[15 19]

In contrast, bi-allelic loss-of-function aberrations in *CNTNAP2* are convincingly causative of an autosomal recessive, fully penetrant, severe ID and epilepsy disorder. In 2006, Strauss *et al*[10] reported on nine affected children in the Old Order Amish with a cortical dysplasia focal epilepsy syndrome (MIM#610042), in whom they identified a homozygous single bp deletion (c.3709delG), predicted to result in a premature stop codon. The affected individuals presented with mildly delayed motor and age-appropriate cognitive development and language comprehension until the onset of frequent and intractable seizures within the first 2 years of live. Subsequently, deterioration of speech, learning

and behaviour was noted, resulting in severe cognitive impairment and behavioural anomalies such as hyperactivity, aggressivity and autism. Head circumference was found to be relatively large. Additionally, diminished or absent deep-tendon reflexes as well as cortical dysplasia in MRI examinations in 43% of individuals and neuronal migration anomalies in brain biopsies of three affected probands were noted. In 2008, a related girl with the same mutation and a similar phenotype was reported, with the additional features of hepatomegaly and periventricular leukomalacia.[20] Shortly after, a pair of siblings with an in-frame homozygous deletion of several exons in *CNTNAP2*, and a sporadic patient with a compound heterozygous intragenic in-frame deletion and splice site mutation were reported.[21] These three individuals had severe ID with very limited or complete lack of speech and more mildly delayed motor development, seizures with onset between 4 and 30 months and episodes of hyperbreathing, which meant that they had previously been suspected to have Pitt-Hopkins syndrome.[21] Since then, to our knowledge only two further families with bi-allelic aberrations in *CNTNAP2* have been reported.[22 23] Two siblings from a consanguineous family carried a homozygous deletion of exon 3 and presented with ataxic cerebral palsy, hyporeflexia, severe cognitive impairment and generalised tonic-clonic seizures from age 2 years.[22] In another consanguineous family, two affected siblings carried a homozygous deletion of exons 2 and 3 and presented with epilepsy and language regression within the first 36 months of life and subsequently with severe ID, absent speech, and behavioural anomalies including autism and obesity.[23]

We now report on a further eight individuals from six families with bi-allelic aberrations in *CNTNAP2* and thus further delineate the genotypic and phenotypic spectrum of recessive *CNTNAP2*-related ID and epilepsy disorders.

## INDIVIDUALS AND METHODS

All eight previously unreported individuals from six unrelated families were seen and diagnosed in different genetic or paediatric-neurological centres worldwide. Aberrations in *CNTNAP2* were detected by various approaches. In individual 1, targeted sequencing of *CNTNAP2* was performed after detecting the gene in a homozygous stretch displayed in data of an Affymetrix 6.0 SNP-Array, which previously had been performed for diagnostic chromosomal microarray analysis. Sequencing of the 24 coding exons of *CNTNAP2* (NM _014141) was performed as described previously.[21]

In individuals 2, 3 and 6, diagnostic chromosomal microarray testing was performed by array-comparative genomic hybridisation or SNP-Array. After detecting a heterozygous deletion in individual 2, multiplex ligation-dependent probe amplification (MLPA) analyses for confirmation purposes was performed as described,[21] and the second allele was sequenced as described above and previously.[21] Parental follow-up investigations of the deletion in parents of individual 2 were carried out by FISH studies according to standard procedures, using the RP11-71M9 probe.

In individual 4, sequencing of the affected proband with a gene panel of 565 ID genes was performed as described elsewhere,[24] and the variant was confirmed by Sanger sequencing. Subsequently, CNVs were tested with a high-resolution SNP-Array. Segregation testing was performed by Sanger sequencing and SNP-Array in the proband's affected brother and his unaffected parents.

In individuals 7 and 8, sequencing was performed using a gene panel of 150 encephalopathy-related genes. DNA was amplified using custom Ampliseq (Life Technologies) and then sequenced on an Ion Proton platform (Life Technologies). The presence of the mutation was confirmed by Sanger sequencing and tested in the parents.

Analyses were performed either in a diagnostic or research setting, and informed consent was obtained from parents or guardians of all affected individuals. If done in a research setting, the studies were approved by the ethic committees of the respective universities or centres.

## RESULTS

### Mutational spectrum

An overview on the published and herewith identified novel aberrations in *CNTNAP2* is displayed in figure 1. In individual 1, targeted sequencing of *CNTNAP2* revealed the homozygous mutation c.1480G>T in exon 9, predicted to result in a premature stop-codon, p.(Glu494*). Both non-consanguineous parents were confirmed to be heterozygous carriers. This variant occurs in the Exome Aggregation Consortium (ExAC) browser[25] with a frequency of $8\times10^{-6}$ (1/121 258 alleles).

In individual 2, chromosomal microarray testing detected a heterozygous deletion of exon 1, including the start codon (1.42 Mb; arr[hg19]7q35(144 520 633–145 949 971)×1), which was confirmed by MLPA and FISH analyses and shown to be inherited from the father. Sequencing of the second allele detected the heterozygous variant c.3046C>T in exon 19. This variant was shown to be inherited from the mother and is predicted to result in a premature stop-codon, p.(Arg1016*). This nonsense variant is not contained in the ExAC database.

Diagnostic chromosomal microarray analysis revealed compound heterozygous CNVs in two families. Individual 3 harboured a maternally inherited deletion of exon 1 (56 kb; arr[hg19]7q35(145 795 795–145 824 743)×1) and a paternally inherited deletion of exons 4 to 20 (1.23 Mb; arr[hg19]7q35q36.1(146 730 472–147 928 239)×1). The deletion of exon 1 is predicted to result in loss of the start codon and the loss of exons 4–20 in frameshifting and thus truncation of the protein. In individual 6, chromosomal microarray analysis detected a paternally inherited deletion of exons 2 and 3 (arr[hg19]7q35(146 389 192–146 587 308)×1), and on the other allele a maternally inherited duplication (arr[hg19]7q35(146 328 875–147 256 625)×3), spanning exons 2–11. The deletion of exons 2 and 3 is predicted to result in frameshifting and thus truncation of the protein. The duplication, if in-tandem, would be in-frame but contains exons encoding several functional domains.

In two affected brothers (individuals 4 and 5), panel sequencing revealed the heterozygous variant c.2963delC, inherited from the father and predicted to result in frameshifting and thus truncation of the protein (p.(Cys989Alafs*45)). This variant is not listed in the ExAC database. Subsequent chromosomal microarray analysis showed a heterozygous deletion of exons 9 and 10 (arr[hg19]7q35(146 988 989–147 101 705)×1), inherited from the mother and predicted to result in frameshifting, too.

Panel sequencing in individuals 7 and 8 from the same family revealed the homozygous variant c.2046C>A, predicted to result in a premature stop codon, p.(Cys682*). Parents were consanguineous and each of them confirmed to be heterozygous carrier. This nonsense variant is not contained in the ExAC database.

### Clinical phenotype

Clinical details are displayed in table 1. All eight individuals with bi-allelic aberrations in *CNTNAP2* identified in this study have ID, estimated to be severe in six of them. Expressive

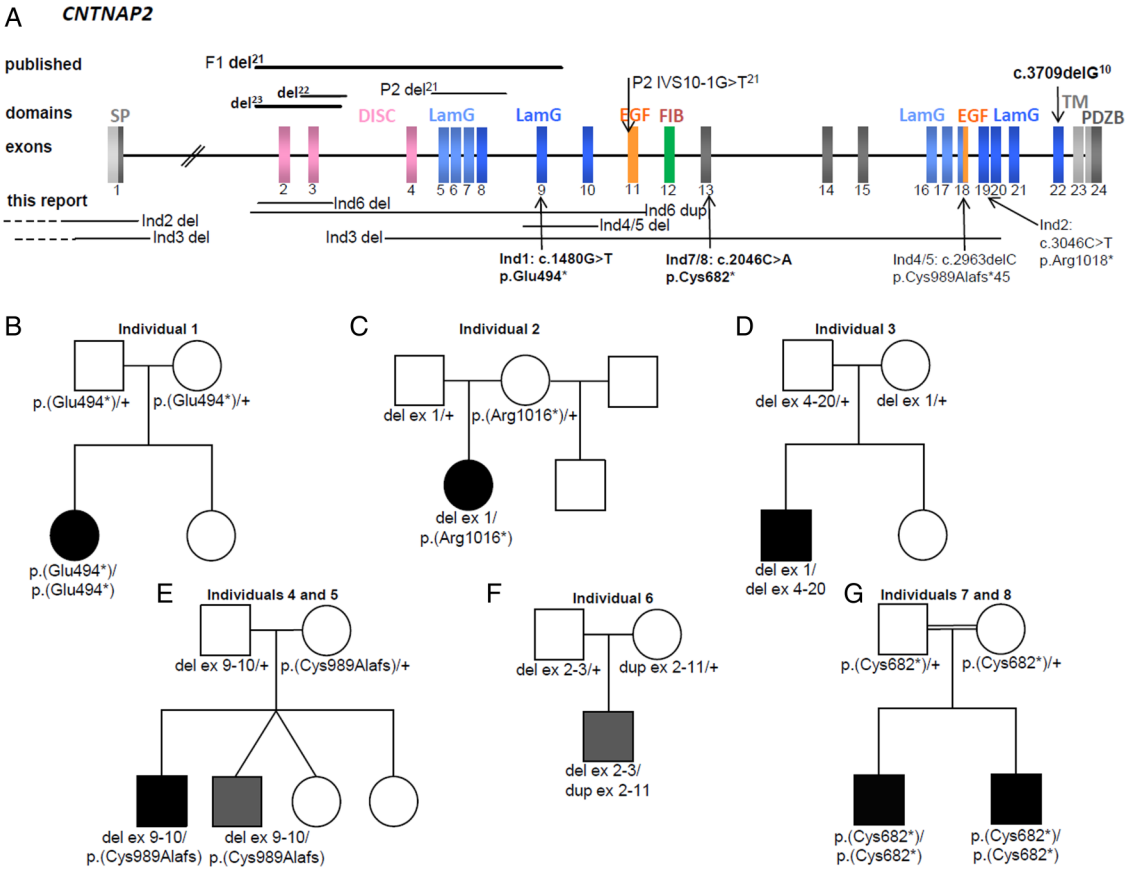


**Figure 1** Structure of *CNTNAP2* and identified aberrations. (A) Schematic drawing of the genomic structure of *CNTNAP2* (NM_014141) with colour coding for domain-coding exons as displayed previously,[21] and localisation of copy number variants and mutations (domains: SP, signal peptide; DISC, discoidin-like domain; LamG, laminin-G domain; EGF, epidermal growth factor-like domain; FIB, fibrinogen-like domain; TM, transmembrane region; PDZPB, PDZ-domain-binding site). Black bars represent deletions or duplications. Bold bars or letters represent homozygous aberrations. References for previously published aberrations are displayed by superscript numbers. Ind, individual(s). (B–G) Pedigrees of the herewith reported individuals with segregation of the identified aberrations in *CNTNAP2*. Black filling indicates severe intellectual disability (ID), dark grey filling moderate ID.

speech is absent or limited to a few single words, and verbal comprehension is very limited as well. Of note, age at speaking first words was appropriate in some of the individuals until loss of verbal skills or stagnation of speech and language development was noted. Two individuals are reported to be intellectually disabled in a moderate range, with the ability to speak in sentences, though simple or with articulation difficulties. One of them had a formally tested IQ of 56 at the age of 11 years. Walking age ranged from 18 months to 5 years in five individuals, and one individual is non-ambulatory at age 14 years. Loss of motor skills is only reported in one individual, another one has progressive difficulties with balance.

Epilepsy occurred in all individuals, and age of onset ranged from 14 months to 3 years 3 months. In two individuals, temporal cortical dysplasia was suspected from MRI results, but could not be confirmed in one. Unclear white matter hyperintensities were observed in two individuals, and vermian atrophy in one. MRI was reported to be normal in three individuals. Decreased deep tendon reflexes were reported in three individuals, and variably hypotonia, ataxia or spasticity in two individuals each. Behavioural anomalies such as stereotypic hand movements, aggressivity, autoaggressivity or reduced eye contact were noted in five individuals. Birth measurements and postnatal growth appear to be in the normal range in most of the individuals for whom this information was available. One individual was reported to be macrocephalic, another one to be microcephalic. Facial dysmorphism or major malformations did not seem to be common.

## DISCUSSION

*CNTNAP2* and its encoded CASPR2 are widely discussed in the literature due to their crucial role in nervous system development and function and their association with a broad spectrum of neurodevelopmental and neuropsychiatric disorders.[26–28] However, information on bi-allelic aberrations in *CNTNAP2*, that initially led to the gene being implicated in neurodevelopmental disease,[10] and that are causative of a fully penetrant, severe epilepsy and ID disorder, is still limited.[20–23 29] In general, homozygous recessive aberrations in *CNTNAP2* seem to be a rare cause of neurodevelopmental disorders, as they were not detected in a study of 136 consanguineous families[30] or in a group of 150 consanguineous families with ID tested in house (unpublished data). The family of individuals 7 and 8 was the only one with a *CNTNAP2* mutation detected among 700 analyses done on a routine diagnostics basis. Furthermore, in outbred populations with sporadically affected individuals, autosomal dominant de novo genetic aberrations were shown to be more frequently the cause of ID than recessive conditions.[31–33]

We have now considerably broadened the mutational and clinical spectrum by reporting eight individuals from six

**Table 1** Clinical details

| Individuals | Individual 1 | Individual 2 | Individual 3 | Individual 4* | Individual 5* | Individual 6 | Individual 7* | Individual 8* |
|---|---|---|---|---|---|---|---|---|
| Gender | Female | Female | Male | Male | Male | Male | Male | Male |
| Age at last investigation | 12 years 5 months | 2 years | 4 years 7 months | 47 years | 40 years | 13 years | 14 years 2 months | 9 years 8 months |
| Genetic aberration | c.1480G>T, hom (mat +pat) | del exon 1, het (pat)+c.3046C>T, het (mat) | del exon 1, het (mat)+del exons 4–20, het (pat) | del exons 9 and 10, het (pat)+c.2963delC, het (mat) | del exons 9 and 10, het (pat)+c.2963delC, het (mat) | del exons 2 and 3, het (pat)+dup exons 2–11, het (mat) | c.2046C>A, hom (mat+pat) | c.2046C>A, hom (mat+pat) |
| Predicted effect on protein | p.(Glu494*) | LSC+p.(Arg1016*) | LSC+fs | fs+p.(Cys989Alafs*45) | fs+p.(Cys989Alafs*45) | fs+in-frame dup | p.(Cys682*) | p.(Cys682*) |
| Detection | Targeted sequencing | arrayCGH, targeted sequencing | arrayCGH | SNP-Array, multigene panel | SNP-Array, multigene panel | arrayCGH | Multigene panel | Multigene panel |
| Parents | Healthy, non-consanguineous | Healthy | Healthy, non-consanguineous | Healthy, non-consanguineous | Healthy, non-consanguineous | Mother: lability, anxiety; non-consanguineous | Healthy, consanguineous | Healthy, consanguineous |
| Gestational age | 39 weeks | nk | 40 weeks | 40 weeks | 38 weeks (dizygotic twin pregnancy) | at term | 39 weeks | 37 weeks |
| Birth weight/SD | 2770 g/−1.37 | nk | 3075 g/−1.24 | 4500 g/1.99 | 3700 g/0.89 | 3850 g/0.51 | 3120 g/−0.84 | 2650 g/−1.02 |
| Birth length/SD | 51 cm/−0.05 | nk | 48 cm/−1.96 | nk | nk | nk | 49 cm/−1.26 | 45 cm/−2.17 |
| Birth OFC/SD | ? | nk | 36 cm/0.31 | nk | nk | nk | nk | 32.5 cm/−1.27 |
| Height/SD | 153 cm/−0.56 | nk | 108.6 cm/0.02 | 164 cm/−2.16 | 167 cm/−1.74 | 163 cm/0.06 | −1.2 | −0.6 |
| Weight/SD | 76.8 kg/1.92 | nk | 19.9 kg/1.25 | 62.5 kg/BMI 23.2 | 80.5 kg/BMI 28.9 | 72.9 kg/1.64 | −2.3 | −0.4 |
| OFC/SD | 57 cm/1.85 | 9th ct. | 50.0/−0.95 | 57 cm/0.09 | 59 cm/1.32 | 55.5 cm/0.31 | −2.6 | −0.3 |
| Walking at age | 24 months | 3 years | 18 months | 18 months–2 years | 2–3 years | 2 years | No | 5 years |
| First words at age | 12 months | nk | 2 years | 12–15 months | 2–3 years | 1 years, delay | 18 months | no |
| Loss of verbal skills, at age | Yes, 2 years | Yes, 14 months | No loss | Yes, 18 months | No loss | No loss | Stagnation, 18 months | Stagnation, 2 years |
| Speech abilities at last investigation | No words, babbling | No words with 3 years, babbling | 10 words, few 2-word sentences | Non-verbal, gestures | Sentences, articulation difficulties | Verbal, simple sentences | No words | No words |
| Comprehension | Limited | nk | Yes | Very limited | 2–3 stage instructions | Limited | Extremely limited | Very limited |
| Purposeful hand use | Yes | nk | Yes | Yes, can use fork and spoon | Yes, can write simple sentences | Yes | No | Yes |
| Best motor function at age/ loss of skills | Progressive balance problems | nk | Driving trainer bike 4 years/ no loss | Could run and climb but not ride a bike, walks with bent knees/no loss | Could ride bike with trainer wheels in childhood/no loss | Constant evolution | 18 months/then loss | Currently/no loss |
| ID | Severe, no formal test | Severe | Severe, no formal test | Severe, no formal test | Moderate (basic literacy skills) | Moderate (IQ56; 11 years, Leiter non-verbal) | Severe, no formal test | Severe, no formal test |
| Seizures/age of onset | Complex focal/2 years | Yes/14 months | Focal tonic, complex focal/ 17 months | Temporal and generalised/ 18 months | Daily, complex partial/ 15 months | Status epilepticus/3 years 3 months; 3–4/year until 11 years | Yes/2 years 5 months | Yes/2 years 5 months |
| EEG anomalies | Generalised slowing | Focal seizure disorder | Occasional spikes and slowing left temporal | Diffuse cerebral dysfunction | Frequent left frontotemporal epileptiform discharges | Slow, no inter ictal epileptic activity | Slow rhythm, sometimes epileptic discharges | Slow rhythm, sometimes epileptic discharges |

Continued

**Table 1** Continued

| Individuals | Individual 1 | Individual 2 | Individual 3 | Individual 4* | Individual 5* | Individual 6 | Individual 7* | Individual 8* |
|---|---|---|---|---|---|---|---|---|
| Antiepileptic treatment | Oxcarbazepine, valproate | nk | Lamotrigine, valproate (levetiracetam, oxcarpazepine, valproate previously) | Valproate, lamotrigine | Carbamazepine, phenobarbitone (weaning) levetiracetam, zonisamide (starting) | Carbamazepine, topiramate | Lamotrigine (valproat and topiramate previously) | Lamotrigine (valproate previously) |
| Response to treatment | Difficult in the beginning, now ca. 1 seizure per 2 years | nk | Seizure free from age 2 years 5 months | Seizure free for several years | Daily seizures | Improvement at 11 years with topiramate | Yes, persistence of some seizures | Yes |
| MRI anomalies | Mild anomaly in corticomedullary differentiation, no dysplasia confirmed | No | Suspected focal cortical dysplasia left temporal, patchy T2-hyperintensities in white matter right frontal | No MRI | Deep white matter intensities of uncertain clinical significance | No (7 years) | Vermian atrophy | No |
| Deep tendon reflexes | Not tested | Hyporeflexia | Normal | Normal | Hyporeflexia lower limbs | Normal | Hyporeflexia | Normal |
| Neurological anomalies | Hypotonia | | No | Episodic ataxia (acetazolamide) | Pes cavus, nystagmus | | Spasticity lower limbs | Neonatal hypotonia, ataxia |
| Abnormal breathing pattern | Not reported | Episodic hyperventilation | No | Not reported | Not reported | No | Not reported | Not reported |
| Behavioural anomalies | Little eye contact, sometimes aggressive or autoaggressive | Hand wringing | Temper tantrums | Tactile defensiveness, reduced eye contact, skin picking, aggression, self-mutilation (olanzapine) | No, very sociable | Low frustration tolerance, temper tantrums (aripiprazole, trazodone) | Stereotypic hand movements | Stereotypies |
| Facial dysmorphism | Coarse face | No | No | No | No | Mild hypertel., downslanting palp. fissures | No | No |
| Other anomalies | Pubertas precox, pre-axial polydactyly right hand | | No | Constipation, spina bifida occulta, kyphosis, osteoporosis, subclinical hypothyroidism | Initial poor weight gain until tonsillectomy 2 years | | Feeding difficulties | |

*Individuals 4 and 5 as well as individuals 7 and 8 are brothers.
arrayCGH, array-comparative genomic hybridisation; BMI, body mass index; ct, centile; fs, frameshift; het, heterozygous; hom, homozygous; hypertel., hypertelorism; ID, intellectual disability; LSC, loss of start codon; mat, maternal; nk, not known/data not available; palp., palpebral; pat, paternal.

unrelated families with novel bi-allelic aberrations in *CNTNAP2*. Only a deletion of exons 2 and 3, identified compound heterozygously with a duplication in individual 6, was recently reported in a homozygous state in two affected siblings.[23] We identified homozygous point mutations in two families, one of them with two affected brothers and consanguineous parents. The other index individuals harboured compound heterozygous CNVs or CNVs and mutations. In accordance with previously identified aberrations,[10 21–23] all deletions or point mutations are predicted to be deleterious by either truncating the protein or resulting in nonsense-mediated mRNA decay. The duplication of 10 exons in individual 6 is predicted to be in-frame, but affects several protein domains and thus also might severely impair protein function. Interestingly, individual 6, carrying this duplication in combination with a truncating deletion of two exons on the other allele, is rather mildly affected with moderate ID and presence of language abilities. Apart from that, no further possible genotype-phenotype correlation could be deduced. The majority of affected individuals are consistently affected by severe ID. However, interestingly, we observed for the first time significant variability even within a single family. Two brothers, individuals 4 and 5, both carry a compound heterozygous deletion of two exons and a truncating mutation. One index has severe ID and is non-verbal, while his brother is moderately intellectually disabled, with basic literacy skills, speaking sentences and being able to follow 2–3 stage instructions.

Recently, the pathogenic role of both common and rare heterozygous single nucleotide variants, particularly missense variants, in *CNTNAP2* as risk factors for autism spectrum disorders has been debated.[34–36] Although all of the herewith identified aberrations in *CNTNAP2* are predicted to be deleterious, the penetrance in heterozygous carriers seems to be low. Parents of the affected individuals, apart from one with emotional lability and anxiety, were reported to be healthy, of normal intelligence and without significant mental health conditions. However, no specific or detailed clinical and/or psychiatric examinations were performed, so we cannot exclude the possibility of subtle neurobehavioural differences.

Of note, none of the herewith reported individuals was tested for *CNTNAP2* aberrations based on clinical suspicion. CNVs or mutations were identified either after noting the gene in a stretch of homozygosity or by chromosomal microarray analysis or multigene panel sequencing. The first report on a recessive mutation in *CNTNAP2* indicated a rather specific phenotype of cortical dysplasia and focal epilepsy.[10] All 25 affected individuals to date (8 plus 17 published[10 20–23 29]) show epilepsy within the first 3.5 years of life. In contrast to previous reports on intractable seizures,[10 23 37] response to antiepileptic treatment with a range of drugs has been reported as good or satisfactory in most of the affected in this series. Both individuals with complete control of seizures (I3 and I4) were treated with a combination of valproic acid and lamotrigine. Also lack or loss of initially normal speech development and manifestation of behavioural anomalies seem to be frequent, while motor development and abilities appear to be commonly more mildly impaired. Loss of speech seems to correlate with onset of seizures, but not necessarily with the severity or frequency of seizures, as individual 5 with daily seizures even in adulthood is able to speak in sentences and has simple writing abilities. Focal cortical dysplasia was reported in 43% of the initial nine children,[10] but not in any of the subsequently published eight individuals[20–23] and only suspected in one of the herewith reported eight individuals. It might therefore not be as common in *CNTNAP2*-related disorders as assumed previously. White matter or cerebellar or other minor anomalies in MRI examinations are reported in several individuals, but cannot be considered as specific features. Head circumferences were reported to be rather large in the initial group of individuals,[10] but in the subsequently published[20–23] and in our new series of individuals, only one individual is macrocephalic. Some of the characteristic aspects in the initial nine individuals from the Old Order Amish[10] might be linked to a specific effect of the identical, shared mutation and/or similarities of the genetic background. In individuals without cortical dysplasia on MRI or decreased deep tendon reflexes, it might be rather difficult to distinguish the clinical presentation from other epileptic encephalopathies such as *SCN1A*-related (EIEE6, MIM#607208), *SCN2A*-related (EIEE11, MIM#613721) or *PCDH19*-related (EIEE9, MIM#300088) disorders, which also include early onset seizures, developmental regression and lack of or very limited speech development. As microcephaly appears to be rare in individuals with recessive *CNTNAP2* aberrations, this might be an important aspect to distinguish the clinical presentation from Rett syndrome (MIM#312750) that shares developmental regression, loss of speech, behavioural anomalies and seizures. Episodic hyperbreathing that had led to the clinical suspicion of Pitt-Hopkins syndrome in three of the previously published individuals,[21] was only observed once in this series.

Expression of *CNTNAP2* is regulated by FOXP2,[38] a forkhead box transcription factor and the first gene implicated in a specific speech and language disorder.[39] Therefore, a direct role of *CNTNAP2* in speech and language development and impairment has been discussed broadly.[38 40–45] Most of the individuals harbouring bi-allelic aberrations in *CNTNAP2* have lack of speech development or loss of speech abilities. As this, however, appears to correlate with a more general deterioration also of cognition and behaviour after onset of epilepsy, a specific speech and language phenotype in these individuals is difficult to evaluate. Age of first words was reported to be normal in several individuals, and in the two affected with rather good language abilities, apart from articulation difficulties in one, no specific speech defects such as dyspraxia or stuttering were noted.

To summarise, we report on eight individuals from six unrelated families with moderate-to-severe ID, early onset epilepsy and behavioural anomalies, in whom we identified novel bi-allelic aberrations in *CNTNAP2*. We further delineated the mutational and clinical spectrum associated with this gene.

## URLS

ExAC, http://exac.broadinstitute.org/
OMIM, http://www.ncbi.nlm.nih.gov/omim
UCSC genome browser, http://genome.ucsc.edu/

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## REFERENCES

1 Nakabayashi K, Scherer SW. The human contactin-associated protein-like 2 gene (CNTNAP2) spans over 2 Mb of DNA at chromosome 7q35. *Genomics* 2001;73:108–12.
2 Bellen HJ, Lu Y, Beckstead R, Bhat MA. Neurexin IV, caspr and paranodin—novel members of the neurexin family: encounters of axons and glia. *Trends Neurosci* 1998;21:444–9.
3 Poliak S, Gollan L, Martinez R, Custer A, Einheber S, Salzer JL, Trimmer JS, Shrager P, Peles E. Caspr2, a new member of the neurexin superfamily, is localized at the juxtaparanodes of myelinated axons and associates with K+ channels. *Neuron* 1999;24:1037–47.
4 Poliak S, Salomon D, Elhanany H, Sabanay H, Kiernan B, Pevny L, Stewart CL, Xu X, Chiu SY, Shrager P, Furley AJ, Peles E. Juxtaparanodal clustering of Shaker-like K+ channels in myelinated axons depends on Caspr2 and TAG-1. *J Cell Biol* 2003;162:1149–60.
5 Abrahams BS, Tentler D, Perederiy JV, Oldham MC, Coppola G, Geschwind DH. Genome-wide analyses of human perisylvian cerebral cortical patterning. *Proc Natl Acad Sci USA* 2007;104:17849–54.
6 Alarcón M, Abrahams BS, Stone JL, Duvall JA, Perederiy JV, Bomar JM, Sebat J, Wigler M, Martin CL, Ledbetter DH, Nelson SF, Cantor RM, Geschwind DH. Linkage, association, and gene-expression analyses identify CNTNAP2 as an autism-susceptibility gene. *Am J Hum Genet* 2008;82:150–9.
7 Arroyo EJ, Xu T, Poliak S, Watson M, Peles E, Scherer SS. Internodal specializations of myelinated axons in the central nervous system. *Cell Tissue Res* 2001;305:53–66.
8 Anderson GR, Galfin T, Xu W, Aoto J, Malenka RC, Südhof TC. Candidate autism gene screen identifies critical role for cell-adhesion molecule CASPR2 in dendritic arborization and spine development. *Proc Natl Acad Sci USA* 2012;109:18120–5.
9 Peñagarikano O, Abrahams BS, Herman EI, Winden KD, Gdalyahu A, Dong H, Sonnenblick LI, Gruver R, Almajano J, Bragin A, Golshani P, Trachtenberg JT, Peles E, Geschwind DH. Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits. *Cell* 2011;147:235–46.
10 Strauss KA, Puffenberger EG, Huentelman MJ, Gottlieb S, Dobrin SE, Parod JM, Stephan DA, Morton DH. Recessive symptomatic focal epilepsy and mutant contactin-associated protein-like 2. *N Engl J Med* 2006;354:1370–7.
11 Arking DE, Cutler DJ, Brune CW, Teslovich TM, West K, Ikeda M, Rea A, Guy M, Lin S, Cook EH, Chakravarti A. A common genetic variant in the neurexin superfamily member CNTNAP2 increases familial risk of autism. *Am J Hum Genet* 2008;82:160–4.
12 Bakkaloglu B, O'Roak BJ, Louvi A, Gupta AR, Abelson JF, Morgan TM, Chawarska K, Klin A, Ercan-Sencicek AG, Stillman AA, Tanriover G, Abrahams BS, Duvall JA, Robbins EM, Geschwind DH, Biederer T, Gunel M, Lifton RP, State MW. Molecular cytogenetic analysis and resequencing of contactin associated protein-like 2 in autism spectrum disorders. *Am J Hum Genet* 2008;82:165–73.
13 Chiocchetti AG, Kopp M, Waltes R, Haslinger D, Duketis E, Jarczok TA, Poustka F, Voran A, Graab U, Meyer J, Klauck SM, Fulda S, Freitag CM. Variants of the CNTNAP2 5′ promoter as risk factors for autism spectrum disorders: a genetic and functional approach. *Mol Psychiatry* 2015;20:839–49.
14 Friedman JI, Vrijenhoek T, Markx S, Janssen IM, van der Vliet WA, Faas BH, Knoers NV, Cahn W, Kahn RS, Edelmann L, Davis KL, Silverman JM, Brunner HG, van Kessel AG, Wijmenga C, Ophoff RA, Veltman JA. CNTNAP2 gene dosage variation is associated with schizophrenia and epilepsy. *Mol Psychiatry* 2008;13:261–6.
15 Gregor A, Albrecht B, Bader I, Bijlsma EK, Ekici AB, Engels H, Hackmann K, Horn D, Hoyer J, Klapecki J, Kohlhase J, Maystadt I, Nagl S, Prott E, Tinschert S, Ullmann R, Wohlleber E, Woods G, Reis A, Rauch A, Zweier C. Expanding the clinical spectrum associated with defects in CNTNAP2 and NRXN1. *BMC Med Genet* 2011;12:106.
16 Mefford HC, Muhle H, Ostertag P, von Spiczak S, Buysse K, Baker C, Franke A, Malafosse A, Genton P, Thomas P, Gurnett CA, Schreiber S, Bassuk AG, Guipponi M, Stephani U, Helbig I, Eichler EE. Genome-wide copy number variation in epilepsy: novel susceptibility loci in idiopathic generalized and focal epilepsies. *PLoS Genet* 2010;6:e1000962.
17 Petrin AL, Giacheti CM, Maximino LP, Abramides DV, Zanchetta S, Rossi NF, Richieri-Costa A, Murray JC. Identification of a microdeletion at the 7q33-q35 disrupting the CNTNAP2 gene in a Brazilian stuttering case. *Am J Med Genet A* 2010;152A:3164–72.
18 Verkerk AJ, Mathews CA, Joosse M, Eussen BH, Heutink P, Oostra BA, Tourette Syndrome Association International Consortium for G. CNTNAP2 is disrupted in a family with Gilles de la Tourette syndrome and obsessive compulsive disorder. *Genomics* 2003;82:1–9.
19 Poot M, Beyer V, Schwaab I, Damatova N, Van't Slot R, Prothero J, Holder SE, Haaf T. Disruption of CNTNAP2 and additional structural genome changes in a boy with speech delay and autism spectrum disorder. *Neurogenetics* 2010;11:81–9.
20 Jackman C, Horn ND, Molleston JP, Sokol DK. Gene associated with seizures, autism, and hepatomegaly in an Amish girl. *Pediatr Neurol* 2009;40:310–13.
21 Zweier C, de Jong EK, Zweier M, Orrico A, Ousager LB, Collins AL, Bijlsma EK, Oortveld MA, Ekici AB, Reis A, Schenck A, Rauch A. CNTNAP2 and NRXN1 are mutated in autosomal-recessive Pitt-Hopkins-like mental retardation and determine the level of a common synaptic protein in Drosophila. *Am J Hum Genet* 2009;85:655–66.
22 Watson CM, Crinnion LA, Tzika A, Mills A, Coates A, Pendlebury M, Hewitt S, Harrison SM, Daly C, Roberts P, Carr IM, Sheridan EG, Bonthron DT. Diagnostic whole genome sequencing and split-read mapping for nucleotide resolution breakpoint identification in CNTNAP2 deficiency syndrome. *Am J Med Genet A* 2014;164A:2649–55.
23 Rodenas-Cuadrado P, Pietrafusa N, Francavilla T, La Neve A, Striano P, Vernes SC. Characterisation of CASPR2 deficiency disorder—a syndrome involving autism, epilepsy and language impairment. *BMC Med Genet* 2016;17:8.
24 Grozeva D, Carss K, Spasic-Boskovic O, Tejada MI, Gecz J, Shaw M, Corbett M, Haan E, Thompson E, Friend K, Hussain Z, Hackett A, Field M, Renieri A, Stevenson R, Schwartz C, Floyd JA, Bentham J, Cosgrove C, Keavney B, Bhattacharya S, Italian XlMRP, Consortium UK, Consortium G, Hurles M, Raymond FL. Targeted next-generation sequencing analysis of 1,000 individuals with intellectual disability. *Hum Mutat* 2015;36:1197–204.
25 Lek M, Karczewski K, Minikel E, Samocha K, Banks E, Fennell T, O'Donnell-Luria A, Ware J, Hill A, Cummings B, Tukiainen T, Birnbaum D, Kosmicki J, Duncan L, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Cooper D, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki M, Levy Moonshine A, Natarajan P, Orozco L, Peloso G, Poplin R, Rivas M, Ruano-Rubio V, Ruderfer D, Shakir K, Stenson P, Stevens C, Thomas B, Tiao G, Tusie-Luna M, Weisburd B, Won H-H, Yu D, Altshuler D, Ardissino D, Boehnke M, Danesh J, Roberto E, Florez J, Gabriel S, Getz G, Hultman C, Kathiresan S, Laakso M, McCarroll S, McCarthy M, McGovern D, McPherson R, Neale B, Palotie A, Purcell S, Saleheen D, Scharf J, Sklar P, Patrick S, Tuomilehto J, Watkins H, Wilson J, Daly M, MacArthur D. Analysis of protein-coding genetic variation in 60,706 humans. *bioRxiv*. Published Online First: 30 October 2015. doi:10.1101/030338
26 Peñagarikano O, Geschwind DH. What does CNTNAP2 reveal about autism spectrum disorder? *Trends Mol Med* 2012;18:156–63.
27 Poot M. Connecting the CNTNAP2 Networks with Neurodevelopmental Disorders. *Mol Syndromol* 2015;6:7–22.
28 Rodenas-Cuadrado P, Ho J, Vernes SC. Shining a light on CNTNAP2: complex functions to complex disorders. *Eur J Hum Genet* 2014;22:171–8.
29 Zweier C. Severe intellectual disability associated with recessive defects in CNTNAP2 and NRXN1. *Mol Syndromol* 2012;2:181–5.
30 Najmabadi H, Hu H, Garshasbi M, Zemojtel T, Abedini SS, Chen W, Hosseini M, Behjati F, Haas S, Jamali P, Zecha A, Mohseni M, Puttmann L, Vahid LN, Jensen C, Moheb LA, Bienek M, Larti F, Mueller I, Weissmann R, Darvish H, Wrogemann K, Hadavi V, Lipkowitz B, Esmaeeli-Nieh S, Wieczorek D, Kariminejad R, Firouzabadi SG, Cohen M, Fattahi Z, Rost I, Mojahedi F, Hertzberg C, Dehghan A, Rajab A, Banavandi MJ, Hoffer J, Falah M, Musante L, Kalscheuer V, Ullmann R, Kuss AW, Tzschach A, Kahrizi K, Ropers HH. Deep sequencing reveals 50 novel genes for recessive cognitive disorders. *Nature* 2011;478:57–63.
31 de Ligt J, Willemsen MH, van Bon BW, Kleefstra T, Yntema HG, Kroes T, Vulto-van Silfhout AT, Koolen DA, de Vries P, Gilissen C, del Rosario M, Hoischen A, Scheffer H, de Vries BB, Brunner HG, Veltman JA, Vissers LE. Diagnostic exome sequencing in persons with severe intellectual disability. *N Engl J Med* 2012;367:1921–9.
32 Rauch A, Wieczorek D, Graf E, Wieland T, Endele S, Schwarzmayr T, Albrecht B, Bartholdi D, Beygo J, Di Donato N, Dufke A, Cremer K, Hempel M, Horn D, Hoyer J, Joset P, Ropke A, Moog U, Riess A, Thiel CT, Tzschach A, Wiesener A, Wohlleber E, Zweier C, Ekici AB, Zink AM, Rump A, Meisinger C, Grallert H, Sticht H, Schenck A,

Engels H, Rappold G, Schrock E, Wieacker P, Riess O, Meitinger T, Reis A, Strom TM. Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. *Lancet* 2012;380:1674–82.
33 Vissers LE, de Ligt J, Gilissen C, Janssen I, Steehouwer M, de Vries P, van Lier B, Arts P, Wieskamp N, del Rosario M, van Bon BW, Hoischen A, de Vries BB, Brunner HG, Veltman JA. A de novo paradigm for mental retardation. *Nat Genet* 2010;42:1109–12.
34 Jonsson L, Zettergren A, Pettersson E, Hovey D, Anckarsater H, Westberg L, Lichtenstein P, Lundstrom S, Melke J. Association study between autistic-like traits and polymorphisms in the autism candidate regions RELN, CNTNAP2, SHANK3, and CDH9/10. *Mol Autism* 2014;5:55.
35 Murdoch JD, Gupta AR, Sanders SJ, Walker MF, Keaney J, Fernandez TV, Murtha MT, Anyanwu S, Ober GT, Raubeson MJ, DiLullo NM, Villa N, Waqar Z, Sullivan C, Gonzalez L, Willsey AJ, Choe SY, Neale BM, Daly MJ, State MW. No evidence for association of autism with rare heterozygous point mutations in Contactin-Associated Protein-Like 2 (CNTNAP2), or in Other Contactin-Associated Proteins or Contactins. *PLoS Genet* 2015;11:e1004852.
36 Sampath S, Bhat S, Gupta S, O'Connor A, West AB, Arking DE, Chakravarti A. Defining the contribution of CNTNAP2 to autism susceptibility. *PLoS ONE* 2013;8:e77906.
37 Orrico A, Galli L, Zappella M, Lam CW, Bonifacio S, Torricelli F, Hayek G. Possible case of Pitt-Hopkins syndrome in sibs. *Am J Med Genet* 2001;103:157–9.
38 Vernes SC, Newbury DF, Abrahams BS, Winchester L, Nicod J, Groszer M, Alarcon M, Oliver PL, Davies KE, Geschwind DH, Monaco AP, Fisher SE. A functional genetic link between distinct developmental language disorders. *N Engl J Med* 2008;359:2337–45.
39 Lai CS, Fisher SE, Hurst JA, Vargha-Khadem F, Monaco AP. A forkhead-domain gene is mutated in a severe speech and language disorder. *Nature* 2001;413:519–23.
40 Centanni TM, Sanmann JN, Green JR, Iuzzini-Seigel J, Bartlett C, Sanger WG, Hogan TP. The role of candidate-gene CNTNAP2 in childhood apraxia of speech and specific language impairment. *Am J Med Genet B Neuropsychiatr Genet* 2015;168:536–43.
41 Condro MC, White SA. Distribution of language-related Cntnap2 protein in neural circuits critical for vocal learning. *J Comp Neurol* 2014;522:169–85.
42 Newbury DF, Fisher SE, Monaco AP. Recent advances in the genetics of language impairment. *Genome Med* 2010;2:6.
43 Newbury DF, Paracchini S, Scerri TS, Winchester L, Addis L, Richardson AJ, Walter J, Stein JF, Talcott JB, Monaco AP. Investigation of dyslexia and SLI risk variants in reading- and language-impaired subjects. *Behav Genet* 2011;41:90–104.
44 Peter B, Raskind WH, Matsushita M, Lisowski M, Vu T, Berninger VW, Wijsman EM, Brkanac Z. Replication of CNTNAP2 association with nonword repetition and support for FOXP2 association with timed Reading and motor activities in a dyslexia family sample. *J Neurodev Disord* 2011;3:39–49.
45 Whitehouse AJ, Bishop DV, Ang QW, Pennell CE, Fisher SE. CNTNAP2 variants affect early language development in the general population. *Genes Brain Behav* 2011;10:451–6.