

REVIEW

Shining a light on *CNTNAP2*: complex functions to complex disorders

Pedro Rodenas-Cuadrado¹, Jose Ho^{1,2} and Sonja C Vernes^{*,1,3}

The genetic basis of complex neurological disorders involving language are poorly understood, partly due to the multiple additive genetic risk factors that are thought to be responsible. Furthermore, these conditions are often syndromic in that they have a range of endophenotypes that may be associated with the disorder and that may be present in different combinations in patients. However, the emergence of individual genes implicated across multiple disorders has suggested that they might share similar underlying genetic mechanisms. The *CNTNAP2* gene is an excellent example of this, as it has recently been implicated in a broad range of phenotypes including autism spectrum disorder (ASD), schizophrenia, intellectual disability, dyslexia and language impairment. This review considers the evidence implicating *CNTNAP2* in these conditions, the genetic risk factors and mutations that have been identified in patient and population studies and how these relate to patient phenotypes. The role of *CNTNAP2* is examined in the context of larger neurogenetic networks during development and disorder, given what is known regarding the regulation and function of this gene. Understanding the role of *CNTNAP2* in diverse neurological disorders will further our understanding of how combinations of individual genetic risk factors can contribute to complex conditions.

European Journal of Human Genetics (2014) 22, 171–178; doi:10.1038/ejhg.2013.100; published online 29 May 2013

Keywords: *CNTNAP2*; neurogenetics; language disorder

INTRODUCTION

The Contactin-associated protein-like 2 gene (also known as *CNTNAP2*) contains 24 exons (Figure 1a) spanning 2.3 Mb at chromosome 7q35, making it the largest gene in the genome.¹ Disruptions of the *CNTNAP2* gene have been reported in a number of patients displaying a wide range of severe neurological disorders and association studies have implicated variants within this locus in complex traits including language impairment and autism. Given the breadth of phenotypes and disorders linked to *CNTNAP2*, we aim to integrate clinical, functional, evolutionary, and animal model data to understand the normal functions of *CNTNAP2*, and the neurobiological consequences of its disruption.

CNTNAP2 AND COGNITIVE DISORDERS

Mutations of *CNTNAP2*

The first report of a *CNTNAP2*-related disorder was a family carrying a heterozygous translocation disrupting the coding region (at intron 8) and displaying Tourette syndrome, intellectual disability and obsessive compulsive disorder.² This chromosomal translocation likely disrupted the normal expression of genes located elsewhere in the genome. Since then, other patients have been identified with chromosomal rearrangements affecting multiple genes including *CNTNAP2*, which are likely to confound the effects resulting from *CNTNAP2* mutation^{3–9} (Table 1). However, patients carrying point mutations or microdeletions that affect only the *CNTNAP2* locus have now been reported, which provides insight into the deficit caused specifically by reduction or loss of *CNTNAP2*;^{10–16} (Table 1).

One of the most informative reports of a specific loss of *CNTNAP2* function came from a study of an old-order Amish population in which 13 probands were found to carry the same homozygous point mutation within *CNTNAP2* (3709delG).¹⁴ This change introduced a premature stop codon (I1253X) predicted to produce a non-functional protein.^{14,15} Affected children displayed a complex phenotype characterised by cortical dysplasia-focal epilepsy (CDFE), mild gross motor delay and frequent seizures, following which, patients demonstrated regression of learning ability, language and social behaviours including characteristics of attention deficit hyperactivity disorder (ADHD) and autism.¹⁴

As more patients have been identified, it has become apparent that individuals with mutations affecting only *CNTNAP2* have a similar set of core phenotypes. Most cases present with a combination of four key features; intellectual disability (ID), seizures, autistic characteristics, and language problems. ID in these patients ranges from absent to severe. Similarly, autistic characteristics can vary from full-blown autism, to broad spectrum or the presence of some autistic features, such as repetitive behaviour. The majority of these patients display some kind of language impairment, such as dysarthric language, language delay or absent speech/language. Seizures are regularly present and in some cases phenotypes may be secondary to the seizures. Other cognitive disorders that have been found in patients carrying *CNTNAP2* disruptions include schizophrenia, developmental delay and ADHD. Table 1 provides a comprehensive list of patients, genotypes, and phenotypes.

Most disruptions of *CNTNAP2* are heterozygous, suggesting that loss of a single allele could be sufficient to cause disorder (Table 1).

¹Language and Genetics Department, Max Planck Institute for Psycholinguistics, Nijmegen, The Netherlands; ²Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; ³Donders Institute for Brain, Cognition and Behaviour, Radboud University, Nijmegen, The Netherlands

*Correspondence: Dr SC Vernes, Language and Genetics Department, Max Planck Institute for Psycholinguistics, Wundtlaan 1, 6525 XD Nijmegen, The Netherlands. Tel: +312 4352 1911; Fax: +31 24 3521213; E-mail: Sonja.vernes@mpi.nl

Received 23 December 2012; revised 25 March 2013; accepted 9 April 2013; published online 29 May 2013

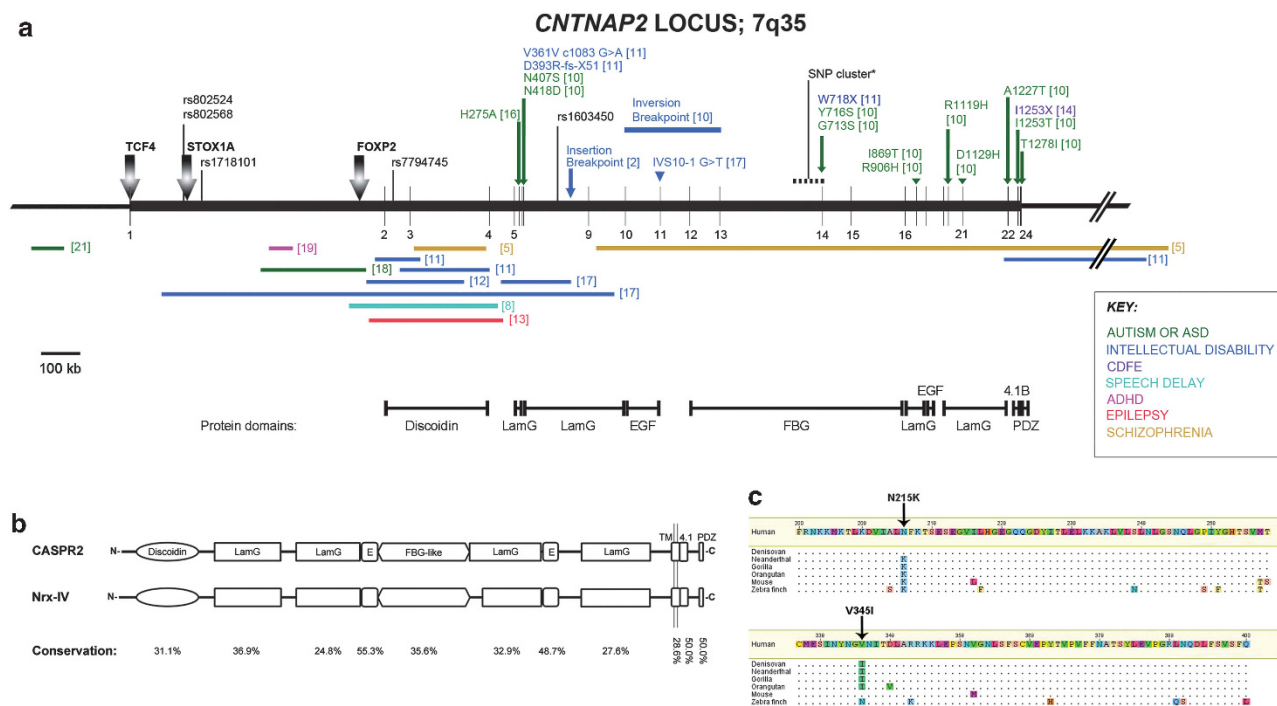


Figure 1 *CNTNAP2* and *CASPR2*. (a) The human *CNTNAP2* locus at 7q35. Schematic indicating the 24 exons (black bars) of the *CNTNAP2* gene. Large grey arrows indicate the experimentally identified binding regions for the transcription factors: TCF4, STOX1A, and FOXP2. Patient identified mutations and deletions as detailed in Table 1 are shown and colour-coded for the primary diagnosis of the patient with references indicated in square brackets. All mutations are heterozygous, except for the CDFE mutation (I1253X) and two Pitt-Hopkins-like ID patients (one homozygous deletion of exons 2–9 and a compound mutation deletion of exons 5–8 plus a frameshift mutation IVS10-1 G>T). The position of the SNPs detailed in Table 2 are indicated by black lines. *The SNP cluster contains the following SNPs: rs851715, rs10246256, rs2710102, rs759178, rs1922892, rs2538991, rs17236239, rs2538976, rs2710117 and rs4431523. The protein domains encoded by each exon are indicated in black below. (b) Domain structure of the human *CASPR2* and *Drosophila* *Nrx-IV* proteins. Despite <50% amino-acid homology between these proteins, the domain structure is very highly conserved (amino-acid similarity is given for each domain of the proteins). Discoidin = Discoidin homology domain, LamG = Laminin G domain, E = EGF-like domain, FBG-like = Fibrinogen-like region, TM = transmembrane domain, 4.1 = Protein 4.1 binding domain, PDZ = PDZ interaction domain. (c) Conservation of *CASPR2*. Human, non-human primate, mouse and Zebra finch amino-acid sequences between amino-acids 200–267 and 336–400. The N215K change was found only in human and denisovan sequences, and the V345I change was found in human and muroid species.

However heterozygous mutations of *CNTNAP2* predicted to be deleterious have also been identified in unaffected individuals. This includes four carriers of the 3709delG (I1253X) mutation in old-order Amish controls¹⁴ and a number of other point mutations in the general population.¹⁰ Thus, the type of mutation present or the genetic background on which the mutations occur may be a major factor in whether a heterozygous disruption can cause disorder. So far only three different homozygous mutations have been identified in patients, all of which cause severe disorder (either CDFE or Pitt-Hopkins-like ID),^{14,17} thus it is possible that complete loss of *CNTNAP2* produces a more severe effect on cognitive function. However, it should be noted that there is evidence that the CDFE mutation may not be a null allele, because it produces a potentially deleterious protein (see section ‘Understanding *CASPR2* function’); thus it will be necessary to identify further patients to clarify this.

Interestingly, three patients carry intronic deletions of *CNTNAP2* not predicted to alter the protein product (Figure 1a).^{5,18,19} An intron 3 deletion containing no known functional elements was identified in a schizophrenia patient who presented with seizures, but normal language.⁵ A deletion within intron 1 was reported in two independent patients suffering from ASD, ID and dysarthric language¹⁸ and from ADHD,¹⁹ respectively. Intron 1 contains a regulatory element bound by the FOXP2 transcription factor.²⁰ Finally, an autistic patient was identified carrying a deletion of an

putative upstream promoter region, which resulted in reduced expression levels.²¹ Thus, although these deletions do not interrupt the protein product, they may affect regulatory elements to produce altered functional levels of *CNTNAP2*.

CNTNAP2 variants are associated with complex disorders

Genome-wide association studies have also linked *CNTNAP2* to complex neurological disorders, including language impairment, autism, dyslexia, schizophrenia, and depression^{18,20,22–26} (Table 2), although causal variants have not yet been identified. Convincing evidence has linked common variants (ie, single nucleotide polymorphisms; SNPs) in the *CNTNAP2* region with the most common form of language disorder in children: specific language impairment (SLI). SLI is diagnosed as abnormal language development in the absence of other medical causes such as hearing loss or autism.²⁷ Significant association was identified for quantitative measures of expressive and receptive language ability as well as scores of non-word repetition (which are thought to relate to phonological working memory and therefore language-related processing ability) with a cluster of SNPs within introns 13–14 (Figure 1a).^{20,24} Non-word repetition was similarly associated with one of these intron 13 variants (rs2710102) in a cohort of dyslexia probands.²⁵

Strong evidence for a connection between *CNTNAP2* and ASD has also emerged. A linkage and association study demonstrated an

Table 1 Patient identified disruptions of CNTNAP2 and associated phenotypes

Primary diagnosis	Other phenotypes	Language	Seizures	Genotype	Proband (Sample size)	References
Autism or ASD	Not specified	Not specified	Not specified	Intron 1 deletion ~200kb	1 (N=1000)	18
	Mental retardation	Dysarthric language	Yes	12 Mb deletion	1 (N=1)	3
	Moderate intellectual disability	Speech delay	Yes	H275A	1 (N=942)	16
	Mild developmental delay, Hyperactivity	Speech delay	No	Complex rearragment.	1 (N=1)	4
	Not specified	Not specified	Not specified	N407S	1 (N=635)	10
	Not specified	Not specified	Not specified	N418D	1 (N=635)	10
	Not specified	Not specified	Not specified	Y716C	1 (N=635)	10
	Not specified	Not specified	Not specified	G731S (conserved)	1 (N=635)	10
	Not specified	Not specified	Not specified	I869T (predicted deleterious)	Four probands, three families (N=635)	10
	Not specified	Not specified	Not specified	R906H	2 (N=635)	10
	Not specified	Not specified	Not specified	R1119H (predicted deleterious)	2 (N=635)	10
	Not specified	Not specified	Not specified	D1129H (predicted deleterious)	2 (N=635)	10
	Not specified	Not specified	Not specified	A1227T	2 (N=635)	10
	Not specified	Not specified	Not specified	I1253T (predicted deleterious)	2 (N=635)	10
	Not specified	Not specified	Not specified	T1278I (predicted deleterious)	2 (N=635)	10
	Not specified	Not specified	Not specified	Promoter deletion ~86 kb	1 (N=41)	21
Intellectual disability	Developmental delay and 'broad spectrum ASD'	Almost absent	No	Inversion, between exons 10–13	1 (N=1)	10
	Severe mental retardation, Autistic features	Almost absent	Yes (epilepsy)	11 Mb deletion	1 (N=1)	5
	Developmental delay, attention deficit, autistic traits	Language delay	Yes (epilepsy)	12.2 Mb deletion	2 sibs (N=2)	6
	Developmental & motor regression (prior to seizure onset) in C3 only	Absent speech	Yes	c.1083G>A (exon 7 splice donor site; pV361V)	2 (N=99)	11
	Motor regression, congenital malformations	Absent speech	Yes	D393R-fs-X51 (frameshift)	1 (N=99)	11
	Not specified	Not specified	Yes	W718X	1 (N=99)	11
	Congenital malformations	Absent speech	No	Deletion exons 2–3	1 (N=99)	11
	Not specified	Absent speech	Yes	Deletion exons 3–4	1 (N=99)	11
	Not specified	Simple speech only	No	Deletion exons 21–3'UTR	1 (N=99)	11
	Pitt–Hopkins like ID	Absent speech	Yes	Deletion exons 2–9. Homozygous	2 sibs (N=179)	17
	Pitt–Hopkins like ID, Autistic behaviour	Absent speech	Yes	Deletion exons 5–8 and exon 11 frameshift mutation IVS10-1 G>T (Compound homozygote)	1 (N=179)	17
Schizophrenia	Developmental delay	Speech problems	No	Deletion exons 2-3	1 (N=8)	12
	Tourette syndrome, some autistic characteristics (OCD, ritualised behaviour)	Speech abnormalities	No	Complex rearragment, intron 8	Father + 2 sibs (N=3)	2
	Congenital malformations	Absent speech	Not specified	Translocation disrupting intron 11	Two family members	9
	Cognitive deterioration	Deteriorated speech, communication difficulties	Yes (rare)	Deletion exon 9–24	1 (case study)	5
Epilepsy	Not specified	Normal	Yes (epilepsy)	Deletion intron 3	1 (N=312)	5
	Not specified	Not specified	Yes	Deletion exon 2–4, plus second deletion (chr17p) affecting other genes	1 (N=517)	13
ADHD	Growth retardation, long QT syndrome	Not specified	Yes (febrile)	12 Mb Deletion	Twins (case study)	7
	Not specified	Not specified	Not specified	Deletion Intron 1	1 (N=335 parent-child trios)	19
CDFE	Intellectual disability, ADHD, ASD	Language regression	Yes (epilepsy)	c.3709delG (I1253X). Homozygous	13 (N=22)	14
	Intellectual disability, ADHD, ASD	Language regression	Yes (epilepsy)	c.3709delG (I1253X). Homozygous	1 (case study)	15
Speech delay	Mild motor delay, reduced orofacial muscle tone	speech delay	No	Deletion exons 2-4	1 (case study)	8

increased familial risk for autism with a SNP in intron 2 of the *CNTNAP2* gene (rs7794745).²³ Independent studies have highlighted significant association between other SNPs and language endophenotypes of ASD, including age at first word (rs2710102)¹⁸ and age at first phrase (rs1718101).²²

Interestingly, a subset of the cluster of SNPs in intron 13–14 (rs2710102; rs759178; rs17236239; rs2538976) have been associated with early communicative behaviour in a large screen of phenotypically normal individuals,²⁸ suggesting that genetic variance at this locus may have a role in individual differences in the general

Table 2 Association studies linking CNTNAP2 SNPs and cognitive function

Primary diagnosis	Quantitative measure	SNP	Sample size	Association	References
Autism	Age at first phrase	rs1718101	1301 families	Additive model of inheritance $P=0.017$	22
	Age at first phrase	rs17236239	1301 families	Recessive model of inheritance $P=0.018$	22
	Age at first word	rs2710102	304 families	Quantitative regression $P=0.028$	18
	Risk of autism	rs7794745	217 families	Transmission disequilibrium test $P=0.00002$	23
SLI	Non-word repetition	rs10246256	184 families, 181 families	QTDT $P=0.001$, QTDT $P=0.0008$	20, 24
	Non-word repetition	rs17236239	184 families, 181 families	QTDT $P=0.00005$, QTDT $P=0.00008$	20, 24
	Non-word repetition	rs2710117	184 families, 181 families	QTDT $P=0.0004$, QTDT $P=0.001$	20, 24
	Non-word repetition	rs2710102	184 families, 181 families	QTDT $P=0.002$, QTDT $P=0.0005$	20, 24
	Non-word repetition	rs851715	184 families	QTDT $P=0.002$	20
	Non-word repetition	rs759178	184 families	QTDT $P=0.002$	20
	Non-word repetition	rs1922892	184 families	QTDT $P=0.002$	20
	Non-word repetition	rs2538991	184 families	QTDT $P=0.002$	20
	Non-word repetition	rs2538976	184 families	QTDT $P=0.002$	20
	Receptive language	rs4431523	184 families	QTDT $P=0.003$	20
	Receptive language (R)	rs10246256	181 families	(R) QTDT $P=0.0032$, (E) QTDT $P=0.0062$	24
	Receptive language (R)	rs17236239	184 families	(R) QTDT $P=0.0336$, (E) QTDT $P=0.0071$	24
	Expressive language (E)	rs2710117	184 families	(R) QTDT $P=0.0056$, (E) QTDT $P=0.0189$	24
Expressive language (E)	rs2710102	184 families	(R) QTDT $P=0.0312$, (E) QTDT $P=0.02$	24	
Dyslexia	Non-word repetition	rs2710102	188 family trios	QTDT $P=0.0174$	25
Schizophrenia	Risk of schizophrenia	rs802524	653 patients	GWAS $P=0.00056$	26
	Risk of schizophrenia	rs802568	653 patients	GWAS $P=0.00089$	26
Bipolar disorder	Risk of bipolar disorder	rs802524	1172 patients	GWAS $P=0.000217$	26
	Risk of bipolar disorder	rs802568	1172 patients	GWAS $P=0.0000138$	26
Normal population	Early communicative behaviour	rs2710102	1149 normal patients	Hypothesis driven GWAS $P=0.0239$	28
	Early communicative behaviour	rs1603450	1149 normal patients	Hypothesis driven GWAS $P=0.0426$	28
	Early communicative behaviour	rs759178	1149 normal patients	Hypothesis driven genome-wide SNP scan $P=0.0248$	28

population. The multiple lines of evidence associating with the rs2710102 SNP provide strong support for contribution of this region to language endophenotypes (Table 2).

Imaging genetics has also implicated SNPs that were previously associated with autism (rs7794745 and rs2710102), and SLI, dyslexia, and communication in normal cohorts (rs2710102).^{29–32} Carrying a 'risk' allele at these loci seems to correlate with altered structural and functional imaging measures. It has been suggested that the effects on structural and functional brain networks seen in individuals carrying these risk variants may underlie their association with disorders such as autism, SLI and schizophrenia. It should be noted, however, that only a handful of studies have been carried out in this area and, as yet, none of these findings have been replicated independently. Further studies with larger cohorts and robust phenotypes will be needed to reveal the true relationship between *CNTNAP2* variants and effects on brain structure and specific aspects of cognitive processing.

MOLECULAR PROPERTIES OF *CNTNAP2*

Taken together, the mutation, association, and imaging data suggest that *CNTNAP2* has an important role in neurocognitive development, but to understand how *CNTNAP2* contributes to both normal development and disorder, it is important to understand the properties of this gene and the product it encodes.

CASPR2

CASPR2, the protein product of *CNTNAP2*, is a member of the neurexin superfamily of proteins that facilitate cell–cell interactions in the nervous system.^{33,34} Specifically, CASPR2 is a single-pass transmembrane protein, distinguished from most other neurexins by an extracellular discoidin/neuropilin homology domain and a fibrinogen-like region (Figure 1b).³⁵ These domains mediate cell–cell adhesions and extracellular matrix interactions.^{36,37} The large extracellular region of CASPR2 also features four laminin G domains and two epidermal growth factor-like (EGF-like) domains predicted to be involved in receptor–ligand interactions, cell adhesion, migration, and differentiation. The small intracellular portion of the protein is largely involved in protein–protein interactions, and contains a type-II PDZ domain and a protein 4.1B binding site (Figure 1b).

Evolution of CASPR2

The CASPR2 protein demonstrates a high degree of conservation amongst mammals, with ~94% amino-acid identity between human and mouse.³⁸ The conservation is even more striking between humans and chimpanzees, where only 6 of the ~1331 residues differ (99.5% identity). An ortholog of *CNTNAP2* has been identified in *Drosophila*, known as *Neurexin-IV* (Nrx-IV) and although amino-acid identity is relatively low (<50%), these proteins display strong conservation of their domain structure (Figure 1b), suggestive of a shared functional role.

Interestingly, recent genome sequencing of an ancient Denisovan DNA sample identified an amino-acid change within CASPR2 (V345I);³⁹ (Figure 1c). In modern humans, this residue (within a Laminin G-like domain) encodes valine, but in non-human primates, Neanderthals and Denisovans, an isoleucine is present (Figure 1c). Subtle changes to proteins such as this may have been important for the evolution of the modern human brain. However, it should be noted that isoleucine and valine share similar amino-acid properties. Moreover, this valine residue is not unique to humans, but is shared with murid species such as mice and rats (Figure 1c). Thus, it is not clear whether this change would have a functional effect. Another nearby amino-acid variant (N215K) may be of evolutionary interest given that in modern humans and Denisovans, this position encodes asparagine. All other species, including Neanderthal, non-human primates, rodents, and songbirds have lysine at this position (Figure 1c). Functional studies are needed to shed light on any effects that may result from these evolutionarily variable amino-acid substitutions.

CNTNAP2 expression pattern

CNTNAP2 is highly expressed throughout the brain and spinal cord. During human brain development, its expression is highest in frontal and anterior lobes, striatum and dorsal thalamus.^{18,40} This expression pattern recapitulates the cortico-striato-thalamic circuitry known to modulate higher order cognitive functions, including speech and language, reward, and frontal executive function. In the human cortex, *CNTNAP2* is expressed in layers II–V¹⁰ with enrichment in Broca's area and other perisylvian brain regions.⁴⁰ The enriched expression of *CNTNAP2* in these brain regions, known to be important for speech and language, is consistent with the emerging role for *CNTNAP2* in normal language development in humans.

In mice, *Cntnap2* expression begins around embryonic day 14 (E14) and is broadly expressed in regions such as the developing cortex and, to a lesser extent, ganglionic eminences.^{40,41} At later developmental stages and in postnatal mice, *Cntnap2* is diffusely expressed in the cortex, striatum, thalamus, hypothalamus, periaqueductal grey and superior colliculus.^{10,40,41} Additionally, *Cntnap2* is strongly expressed in the hippocampal formation in development and in adulthood.^{10,41}

Recently, *Cntnap2* expression has been comprehensively analysed in the Zebra finch brain.⁴² Songbirds have the ability to learn and modify their vocalisations, making them a useful model for studying speech learning and sensorimotor integration. In the songbird, *Cntnap2* shows a highly mosaic pallial expression pattern,⁴² a region with homology to the mammalian cortex.⁴³ However, mammalian cortical layers and songbird pallial nuclei do not show a strict one-to-one correspondence.^{44,45} *Cntnap2* is also expressed in the songbird striatum, thalamus, Purkinje cell layer of the cerebellum and brain regions homologous to the mammalian superior and inferior colliculi.⁴² Strikingly, compared with surrounding tissue, *Cntnap2* expression shows highly differential expression patterns within specialised cortico-striato-thalamic circuitry that makes up the songbird vocal pathway.^{42,46}

Thus, *Cntnap2* expression in the brain appears to be highly conserved between species. However, some differences do occur, mainly, the frontal cortical enrichment in humans that may point to a role in cortical evolution and the development of human cognitive specialisations.

Regulation of CNTNAP2 expression

Little is known about the regulatory programme controlling *CNTNAP2* expression. To date, only four transcription factors have

been shown to regulate *CNTNAP2*; Storkhead box 1A (STOX1A), Transcription factor 4 (TCF4), Forkhead box P2 (FOXP2) and Forkhead box P1 (FOXP1).

STOX1A is a winged-helix transcription factor that is highly expressed in the brain, but its exact expression pattern is yet to be determined. STOX1A regulates *CNTNAP2* by binding to a regulatory region in intron 1 (Figure 1a).⁴⁷ Increasing STOX1A expression in a human neuron-like cell line led to a decrease in both mRNA and protein levels of *CNTNAP2*,⁴⁷ suggesting a repressive role for this transcription factor. However, STOX1A knockdown mildly increased *CNTNAP2* transcript levels without affecting protein levels,⁴⁷ suggesting that *CNTNAP2* protein levels may be controlled by additional mechanisms, such as microRNAs.

TCF4 encodes a basic helix-loop-helix (bHLH) transcription factor that binds near the start site of *CNTNAP2* to upregulate its expression (Figure 1a).⁴⁸ In humans, TCF4 is more highly expressed in the neocortex and hippocampus than in the striatum, thalamus and cerebellum.⁴⁹ Mutations in TCF4 have been shown to cause Pitt-Hopkins syndrome (PTHS) and three rare TCF4 SNPs are associated with schizophrenia.^{17,49–51} PTHS is characterised by severe intellectual disability, absent or severely impaired speech, characteristic facial features and epilepsy.⁵² Many of these features are shared with patients carrying *CNTNAP2* mutations, leading researchers to test patients with PTHS-like features for *CNTNAP2* mutations.¹⁷ Two mutations affecting the *CNTNAP2* locus (one homozygous and one compound heterozygote) were identified in two independent pedigrees (Table 1). This suggested that disruption of the TCF4–*CNTNAP2* pathway could be related to intellectual disability, seizures, and/or behavioural abnormalities.

FOXP2 encodes a transcription factor belonging to the Forkhead-box (FOX) superfamily and is expressed in areas including cortex, striatum, thalamus, and cerebellum. It was originally identified in a large multigenerational pedigree (the KE family) that display difficulties in the production of co-ordinated orofacial movements, developmental verbal dyspraxia and impaired linguistic processing.^{53–55} FOXP2 binds within intron 1 of *CNTNAP2* to regulate gene expression (Figure 1a).²⁰ In neuronal-like cell models and the developing human cortex, an inverse relationship has been observed between FOXP2 and *CNTNAP2* expression,^{20,42,56} supporting the finding that FOXP2 represses *CNTNAP2*.

Another FOXP transcription factor, known as FOXP1, has been shown to downregulate *CNTNAP2*.¹⁶ *FOXP1* and *FOXP2* show highly overlapping expression patterns and can interact to co-regulate gene expression.^{57–60} A single autistic proband was recently identified carrying mutations in both *FOXP1* and *CNTNAP2*, suggesting a potential link between FOXP1–*CNTNAP2* and ASD.¹⁶ This discovery is intriguing given that both *FOXP1* and *CNTNAP2* mutations have been identified in patients with mild-to-moderate ID, in the presence or absence of autistic features.^{61,62}

Combinatorial regulation by these and other factors are likely to be important for the precise control of *CNTNAP2* expression in the developing and adult brain and disruption of these pathways could have very different phenotypic effects. For example, during development (~E14), the activator *Tcf4* is strongly expressed in upper layers of the cortex, and this may contribute to the strong expression of *Cntnap2* in this region. In the developing ganglionic eminences/striatum, *Cntnap2* expression is low, corresponding to high levels of the repressive factors *Foxp1* and *Foxp2*. Thus, mutation of these different factors, or their binding sites within *CNTNAP2* could result in region-specific developmental defects that may relate to observed phenotypes; for example, ID-related phenotypes that have been linked

to *TCF4/CNTNAP2* loss. Temporal-specific effects may also be relevant. At later stages, once the mature cortex has formed, *CNTNAP2* is not expressed in layer-6 neurons, where *FOXP2* expression is highest. Thus, preventing *CNTNAP2* expression in layer-6 of the cortex may be important for neuronal migration and/or function in this region and may have effects, for example, on cortico-thalamic circuitry.

UNDERSTANDING CASPR2 FUNCTION

Although *CNTNAP2* has been studied for many years, the functions of this gene are still poorly understood. Only recently have insights been gained into some of the roles that might be central to its requirement in normal development, and why disruption causes such complex neurological phenotypes.

CASPR2 and the juxtapanode

The most well-defined role for CASPR2 is in the axon initial segment (AIS) and juxtapanodal regions of myelinated nerves,³⁵ where it forms a complex with contactin-2 (known as CNTN2 or TAG-1).^{63,64} Formation of this complex is mediated by Protein 4.1B binding to the intracellular portion of CASPR2 and is required for the clustering of voltage-gated potassium channels at juxtapanodes.⁶⁵ These channels are involved in the rapid, saltatory conduction of nerve impulses,⁶⁶ and individuals from the CDFE cohort (with homozygous *CNTNAP2* mutations) display reduced potassium channel (K_v1.1) localisation in hippocampal axons.¹⁴ However, in *Cntnap2*-knockout mice, no difference in nerve conduction was observed in the peripheral nervous system.^{65,67}

CASPR2 and neuronal migration

Given that *CNTNAP2* expression is high at timepoints prior to the development of neuronal myelination, additional non-juxtapanodal roles for CASPR2 have been proposed. CDFE patients carrying homozygous mutations of *CNTNAP2* displayed histological abnormalities including regions of abnormal cortical thickening, poorly defined grey and white matter junctions and abnormal density and organisation of neurons.¹⁴ In the cortex, hippocampus and amygdala astrocyte density was increased, and ectopic neurons were observed in subcortical white matter and ectopic glia in the cortex,¹⁴ suggesting involvement of CASPR2 in neuronal migration. *Cntnap2*-null mice also display the presence of ectopic neurons in subcortical white matter (corpus callosum) and mislocalisation of neurons within the six-layer cortex, with significant numbers of upper layer neurons found in deeper cortical layers.⁴¹ Reduced numbers of GABAergic (inhibitory) interneurons were found in the cortex, striatum and hippocampus in comparison to wild-type littermates.⁴¹ However, it is not clear if the reduction in interneurons is a migratory defect or due to other mechanisms such as aberrant neurogenesis or neuronal differentiation.

CASPR2 and neuronal networks

Recently, loss of functional CASPR2 has been shown to produce effects at the level of the neural network. CASPR2 knockdown causes decreased dendritic arborisation in mouse cortical neurons,⁶⁸ suggesting a normal role in promoting outgrowth and connectivity. Axonal pathfinding defects were also observed when *Nrx-IV* was absent from *Drosophila* embryos.⁶⁹ In human patients, neuronal morphology, dendrite structure, and orientation were also affected, in keeping with the effects observed in mouse and fly models.¹⁴

Subcellular fractionation studies indicated that CASPR2 and its interaction partner CNTN2 are present at the synapse.¹⁰ Furthermore,

in neuronal cultures, transient Caspr2 knockdown produced decreased amplitude (but not frequency) of synaptic responses, suggesting a cell-autonomous impairment of synaptic transmission.⁶⁸ However, in a mouse model of Caspr2 loss, firing rate and amplitude were not affected. Instead, a reduction of synchronicity of neuronal firing in the somatosensory cortex was detected.⁴¹ These conflicting results may be due to the different experimental setups used, or may reflect developmental differences due to transient *vs* endogenous knockdown of *Cntnap2*. The involvement of Caspr2 in synchronicity of neuronal firing is consistent with the observed effects on inhibitory interneuron function given the role they have in coordination of neural network activity.⁴¹ Interneuron function has also been implicated in disorders such as autism,⁷⁰ schizophrenia,^{71,72} and epilepsy,⁷³ suggesting a possible shared mechanism by which CASPR2 could contribute to these neurological disorders.

Not all of the phenotypes found in the human patients, such as mislocalisation of potassium channels, could be observed in the mouse. This may be due to the knockout of Caspr2 in the mouse model compared with the truncated protein product present in the CDFE patients. This mutation introduces a stop codon (I1253X) that truncates the protein at the transmembrane domain. This results in the expression of a mutant version of CASPR2 containing only the extracellular domains, which is secreted from the cell.⁷⁴ This extracellular version of CASPR2 may be able to interact with other ligands/receptors, and may thus have some additional deleterious effects, rather than representing a null allele.

CASPR2 and behaviour

Mice that lack Caspr2 display an onset of spontaneous seizures at ~6 months of age.⁴¹ Prior to seizure onset, a range of behavioural differences were observed.⁴¹ Knockout mice were significantly more active, with better motor coordination and balance, and were hyper-reactive to thermal stimuli.⁴¹ Other sensory responses such as acoustic and olfactory were not impaired.⁴¹ Given the link between *CNTNAP2*, autism and social communication,^{10,18,28} it is intriguing to note that mutant mice displayed increased stereotyped and repetitive behaviour, and spent less time engaging in social play.⁴¹ Hence, the use of animal models, such as described herein, allow a controlled exploration of the cellular and neurobiological effects of CASPR2 loss. Taking these findings together with patient data, we can start to build a picture of how *CNTNAP2* affects neurodevelopment at a cellular, network and whole-brain level.

CONCLUSIONS

CNTNAP2 mutations produce a complex disorder, yet the core endophenotypes displayed by individuals carrying *CNTNAP2* disruptions are to a degree, consistent. Evidence from patient and animal model data are giving insight into the cause of these disturbances, with CASPR2 implicated in neuronal connectivity at the cellular and network level, interneuron development/function, synaptic organisation and activity and migration of neurons in the developing brain. Different effects of *CNTNAP2* mutation have been observed across species and at different developmental timepoints. It will be of value in the future to separate the developmental from the adult requirements of CASPR2. Moreover to best utilise animal models, it will be necessary to determine which of the aspects of CASPR2 function are human-specific and which are evolutionary conserved. In future, genome-wide analysis of patients to exclude other deleterious changes coupled to coherent and in-depth phenotyping will provide the best

evidence for the specific phenotypes caused by *CNTNAP2* and how these are shared across complex disorders.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

SCV is supported by a Human Frontier Science Program (HFSP) Fellowship. JH is supported by an A*STAR graduate scholarship.

- 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**: 108–112.
- Verkerk AJ, Mathews CA, Jooos M, Eussen BH, Heutink P, Oostra BA: *CNTNAP2* is disrupted in a family with Gilles de la Tourette syndrome and obsessive compulsive disorder. *Genomics* 2003; **82**: 1–9.
- Rossi E, Verri AP, Patricelli MG et al: A 12Mb deletion at 7q33-q35 associated with autism spectrum disorders and primary amenorrhea. *Eur J Med Genet* 2008; **51**: 631–638.
- Poot M, Beyer V, Schwaab I et al: Disruption of *CNTNAP2* and additional structural genome changes in a boy with speech delay and autism spectrum disorder. *Neurogenetics* 2010; **11**: 81–89.
- Friedman JI, Vrijenhoek T, Markx S et al: *CNTNAP2* gene dosage variation is associated with schizophrenia and epilepsy. *Mol Psychiatr* 2008; **13**: 261–266.
- Sehested LT, Møller RS, Bache I et al: Deletion of 7q34-q36.2 in two siblings with mental retardation, language delay, primary amenorrhea, and dysmorphic features. *Am J Med Genet A* 2010; **152A**: 3115–3119.
- Bisgaard A-M, Rackauskaite G, Thelle T, Kirchhoff M, Bryndorf T: Twins with mental retardation and an interstitial deletion 7q34q36.2 leading to the diagnosis of long QT syndrome. *Am J Med Genet A* 2006; **140**: 644–648.
- Al-Murrani A, Ashton F, Aftimos S, George AM, Love DR: Amino-terminal microdeletion within the *CNTNAP2* gene associated with variable expressivity of speech delay. *Case Rep Genet* 2012; **2012**: 172408.
- Belloso JM, Bache I, Guitart M et al: Disruption of the *CNTNAP2* gene in a t(7;15) translocation family without symptoms of Gilles de la Tourette syndrome. *Eur J Hum Genet* 2007; **15**: 711–713.
- Bakkaloglu B, O'Roak BJ, Louvi A et al: Molecular cytogenetic analysis and resequencing of contactin associated protein-like 2 in autism spectrum disorders. *Am J Hum Genet* 2008; **82**: 165–173.
- Gregor A, Albrecht B, Bader I et al: Expanding the clinical spectrum associated with defects in *CNTNAP2* and *NRXN1*. *BMC Med Genet* 2011; **12**: 106.
- Mikhail FM, Lose EJ, Robin NH et al: Clinically relevant single gene or intragenic deletions encompassing critical neurodevelopmental genes in patients with developmental delay, mental retardation, and/or autism spectrum disorders. *Am J Med Genet A* 2011; **155A**: 2386–2396.
- Mefford HC, Muhle H, Ostertag P et al: Genome-wide copy number variation in epilepsy: novel susceptibility loci in idiopathic generalized and focal epilepsies. *PLoS Genet* 2010; **6**: e1000962.
- Strauss K, Puffenberger EG, Huentelman MJ et al: Recessive symptomatic focal epilepsy and mutant contactin-associated protein-like 2. *N Engl J Med* 2006; **354**: 1370–1377.
- Jackman C, Horn ND, Mollleston JP, Sokol DK: Gene associated with seizures, autism, and hepatomegaly in an Amish girl. *Pediatr Neurol* 2009; **40**: 310–313.
- O'Roak BJ, Deriziotis P, Lee C et al: Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations. *Nat Genet* 2011; **43**: 585–589.
- Zweier C, de Jong EK, Zweier M et al: *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**: 655–666.
- Alarcon M, Abrahams BS, Stone JL et al: Linkage, association, and gene-expression analyses identify *CNTNAP2* as an autism-susceptibility gene. *Am J Hum Genet* 2008; **82**: 150–159.
- Elia J, Gai X, Xie HM et al: Rare structural variants found in attention-deficit hyperactivity disorder are preferentially associated with neurodevelopmental genes. *Mol Psychiatr* 2010; **15**: 637–646.
- Vernes SC, Newbury DF, Abrahams BS et al: A functional genetic link between distinct developmental language disorders. *N Engl J Med* 2008; **359**: 2337–2345.
- Nord AS, Roeb W, Dickel DE et al: Reduced transcript expression of genes affected by inherited and de novo CNVs in autism. *Eur J Hum Genet* 2011; **19**: 727–731.
- Anney R, Klei L, Pinto D et al: Individual common variants exert weak effects on the risk for autism spectrum disorders. *Hum Mol Genet* 2012; **21**: 4781–4792.
- Arking DE, Cutler DJ, Brune CW et al: A common genetic variant in the neurexin superfamily member *CNTNAP2* increases familial risk of autism. *Am J Hum Genet* 2008; **82**: 160–164.
- Newbury DF, Paracchini S, Scerri TS et al: Investigation of dyslexia and SLI risk variants in reading- and language-impaired subjects. *Behav Genet* 2011; **41**: 90–104.
- Peter B, Raskind WH, Matsushita M et al: 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.
- Ji W, Li T, Pan Y et al: *CNTNAP2* is significantly associated with schizophrenia and major depression in the Han Chinese population. *Psychiatr Res* 2012 pii **S0165-1781**: 00521–00525.
- Bishop DV: Genetic and environmental risks for specific language impairment in children. *Inter Congress Ser* 2003; **1254**: 225–245.
- Whitehouse aJO, Bishop DVM, Ang QW, Pennell CE, Fisher SE: *CNTNAP2* variants affect early language development in the general population. *Genes Brain Behav* 2012; **11**: 501.
- Scott-Van Zeeland AA, Abrahams BS, Alvarez-Retuerto AI et al: Altered functional connectivity in frontal lobe circuits is associated with variation in the autism risk gene *CNTNAP2*. *Sci Transl Med* 2010; **2**: 56ra80.
- Kos M, van den Brink D, Snijders TM et al: *CNTNAP2* and language processing in healthy individuals as measured with ERPs. *PLoS One* 2012; **7**: e46995.
- Dennis EL, Jahanshad N, Rudie JD et al: Altered structural brain connectivity in healthy carriers of the autism risk gene, *CNTNAP2*. *Brain Connect* 2011; **1**: 447–459.
- Tan GCY, Doke TF, Ashburner J, Wood NW, Frackowiak RSJ: Normal variation in fronto-occipital circuitry and cerebellar structure with an autism-associated polymorphism of *CNTNAP2*. *NeuroImage* 2010; **53**: 1030–1042.
- Missler M, Südhof TC: Neurexophilins form a conserved family of neuropeptide-like glycoproteins. *J Neurosci* 1998; **18**: 3630–3638.
- Dean C, Dresbach T: Neuroligins and neuexins: linking cell adhesion, synapse formation and cognitive function. *Trends Neurosci* 2006; **29**: 21–29.
- Poliak S, Gollan L, Martinez R et al: Caspr2, a new member of the neurexin superfamily, is localized at the juxtaparanodes of myelinated axons and associates with K⁺ channels. *Neuron* 1999; **24**: 1037–1047.
- Bristow J, Tee MK, Gitelman SE, Mellon SH, Miller WL: Tenascin-X: a novel extracellular matrix protein encoded by the human XB gene overlapping P450c21B. *J Cell Biol* 1993; **122**: 265–278.
- Couto JR, Taylor MR, Godwin SG, Ceriani RL, Peterson JA: Cloning and sequence analysis of human breast epithelial antigen BA46 reveals an RGD cell adhesion sequence presented on an epidermal growth factor-like domain. *DNA Cell Biol* 1996; **15**: 281–286.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ: Basic local alignment search tool. *J Mol Biol* 1990; **215**: 403–410.
- Meyer M, Kircher M, Gansauge M-T et al: A high-coverage genome sequence from an archaic Denisovan individual. *Science* 2012; **338**: 222–226.
- Abrahams BS, Tentler DJ, Perederiy V, Oldham MC, Coppola G, Geschwind DH: Genome-wide analyses of human perisylvian cerebral cortical patterning. *Proc Natl Acad Sci USA* 2007; **104**: 17849–17854.
- Peñagarikano O, Abrahams BS, Herman EI et al: Absence of *CNTNAP2* leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits. *Cell* 2011; **147**: 235–246.
- Panaïtof SC, Abrahams BS, Dong H, Geschwind DH, White SA: Language-related *Cntnap2* gene is differentially expressed in sexually dimorphic song nuclei essential for vocal learning in songbirds. *J Compar Neurol* 2010; **518**: 1995–2018.
- Reiner A, Perkel DJ, Bruce LL et al: The Avian Brain Nomenclature Forum: Terminology for a New Century in Comparative Neuroanatomy. *J Compar Neurol* 2004; **473**: e1–e6.
- Karten HJ: Homology and evolutionary origins of the “neocortex”. *Brain Behav Evol* 1991; **38**: 264–272.
- Puelles L, Kuwana E, Puelles E et al: Pallial and subpallial derivatives in the embryonic chick and mouse telencephalon, traced by the expression of the genes *Dlx-2*, *Emx-1*, *Nkx-2.1*, *Pax-6*, and *Tbr-1*. *J Compar Neurol* 2000; **424**: 409–438.
- Scharff C, Nottebohm F: A comparative study of the behavioral deficits following lesions of various parts of the zebra finch song system: implications for vocal learning. *J Neurosci* 1991; **11**: 2896–2913.
- Van Abel D, Michel O, Veerhuis R, Jacobs M, van Dijk M, Oudejans CBM: Direct Downregulation of *CNTNAP2* by *STOX1A* is Associated with Alzheimer's Disease. *J Alzheimer Dis* 2012; **31**: 1–8.
- Forrest M, Chapman RM, Doyle M, Tinsley CL, Waite A, Blake DJ: Functional analysis of TCF4 missense mutations that cause Pitt-Hopkins Syndrome. *Hum Mutat* 2012; **33**: 1676–1686.
- Whalen S, Héron D, Gaillon T et al: Novel comprehensive diagnostic strategy in Pitt-Hopkins syndrome: clinical score and further delineation of the TCF4 mutational spectrum. *Hum Mutat* 2012; **33**: 64–72.
- Stefansson H, Ophoff RA, Steinberg S et al: Common variants conferring risk of schizophrenia. *Nature* 2009; **460**: 744–747.
- Li T, Li Z, Chen P et al: Common variants in major histocompatibility complex region and TCF4 gene are significantly associated with schizophrenia in Han Chinese. *Biol Psychiatr* 2010; **68**: 671–673.
- Peippo M, Ignatius J: Pitt-Hopkins Syndrome. *Mol Syndromol* 2012; **2**: 171–180.
- Vargha-Khadem F, Watkins K, Alcock K, Fletcher P, Passingham R: Praxic and nonverbal cognitive deficits in a large family with a genetically transmitted speech and language disorder. *Proc Natl Acad Sci USA* 1995; **92**: 930–933.
- Watkins KE, Dronkers NF, Vargha-Khadem F: Behavioural analysis of an inherited speech and language disorder: comparison with acquired aphasia. *Brain* 2002; **125**: 452–464.

- 55 Vargha-Khadem F, Watkins KE, Price CJ *et al*: Neural basis of an inherited speech and language disorder. *Proc Natl Acad Sci USA* 1998; **95**: 12695–12700.
- 56 Haesler S, Wada K, Nshdejan A *et al*: FoxP2 expression in avian vocal learners and non-learners. *J Neurosci* 2004; **24**: 3164–3175.
- 57 Li S, Weidenfeld J, Morrisey EE: Transcriptional and DNA binding activity of the Foxp1/2/4 family is modulated by heterotypic and homotypic protein interactions. *Society* 2004; **24**: 809–822.
- 58 Teramitsu I, Kudo LC, London SE, Geschwind DH, White SA: Parallel FoxP1 and FoxP2 expression in songbird and human brain predicts functional interaction. *J Neurosci* 2004; **24**: 3152–3163.
- 59 Lai CSL, Gerrelli D, Monaco AP, Fisher SE, Copp AJ: FOXP2 expression during brain development coincides with adult sites of pathology in a severe speech and language disorder. *Brain* 2003; **126**: 2455–2462.
- 60 Ferland RJ, Cherry TJ, Preware PO, Morrisey EE, Walsh CA: Characterization of Foxp2 and Foxp1 mRNA and protein in the developing and mature brain. *J Compar Neurol* 2003; **460**: 266–279.
- 61 Hamdan FF, Daoud H, Rochefort D *et al*: *De novo* mutations in FOXP1 in cases with intellectual disability, autism, and language impairment. *Am J Hum Genet* 2010; **87**: 671–678.
- 62 Horn D, Kapeller J, Rivera-Brugués N *et al*: Identification of FOXP1 deletions in three unrelated patients with mental retardation and significant speech and language deficits. *Hum Mutat* 2010; **31**: E1851–E1860.
- 63 Inda MC, DeFelipe J, Muñoz A: Voltage-gated ion channels in the axon initial segment of human cortical pyramidal cells and their relationship with chandelier cells. *Proceedings of the National Academy of Sciences of the United States of America* 2006; **103**: 2920–2925.
- 64 Duflocq A, Chareyre F, Giovannini M, Couraud F, Davenne M: Characterization of the axon initial segment (AIS) of motor neurons and identification of a para-AIS and a juxtapara-AIS, organized by protein 4.1B. *BMC Biol* 2011; **9**: 66.
- 65 Poliak S, Salomon D, Elhanany H *et al*: Juxtaparanodal clustering of Shaker-like K⁺ channels in myelinated axons depends on Caspr2 and TAG-1. *J Cell Biol* 2003; **162**: 1149–1160.
- 66 Vabnick I, Trimmer JS, Schwarz TL, Levinson SR, Risal D, Shrager P: Dynamic potassium channel distributions during axonal development prevent aberrant firing patterns. *J Neurosci* 1999; **19**: 747–758.
- 67 Traka M, Dupree JL, Popko B, Karagogeos D: The neuronal adhesion protein TAG-1 is expressed by Schwann cells and oligodendrocytes and is localized to the juxtaparanodal region of myelinated fibers. *J Neurosci* 2002; **22**: 3016–3024.
- 68 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**: 18120–18125.
- 69 Banerjee S, Blauth K, Peters K, Rogers SL, Fanning AS, Bhat MA: Drosophila neurexin IV interacts with Roundabout and is required for repulsive midline axon guidance. *J Neurosci* 2010; **30**: 5653–5667.
- 70 Leviitt P, Eagleson KL, Powell EM: Regulation of neocortical interneuron development and the implications for neurodevelopmental disorders. *Trends Neurosci* 2004; **27**: 400–406.
- 71 Lewis DA, Gonzalez-Burgos G: Intrinsic excitatory connections in the prefrontal cortex and the pathophysiology of schizophrenia. *Brain Res Bull* 2000; **52**: 309–317.
- 72 Benes FM, Berretta S: GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. *Neuropsychopharmacology* 2001; **25**: 1–27.
- 73 Cobos I, Calcagnotto ME, Vilaythong AJ *et al*: Mice lacking Dlx1 show subtype-specific loss of interneurons, reduced inhibition and epilepsy. *Nat Neurosci* 2005; **8**: 1059–1068.
- 74 Falivelli G, De Jaco A, Favaloro FL *et al*: Inherited genetic variants in autism-related CNTNAP2 show perturbed trafficking and ATF6 activation. *Hum Mol Genet* 2012; **21**: 4761–4773.