Comprehensive cross-disorder analyses of CNTNAP2 suggest it is unlikely to be a primary risk gene for psychiatric disorders

Claudio Toma¹,2*, Kerrie D. Pierce¹, Alex D. Shaw¹,2, Anna Heath¹, Philip B. Mitchell³,4, Peter R. Schofield¹,2, Janice M. Fullerton¹,2

¹ Neurosciences Research Australia, Sydney, Australia, ² School of Medical Sciences, University of New South Wales, Sydney, Australia, ³ School of Psychiatry, University of New South Wales, Sydney, Australia, ⁴ Black Dog Institute, Prince of Wales Hospital, Sydney, Australia

* c.toma@neura.edu.au

Abstract

The contactin-associated protein-like 2 (CNTNAP2) gene is a member of the neurexin superfamily. CNTNAP2 was first implicated in the cortical dysplasia-focal epilepsy (CDFE) syndrome, a recessive disease characterized by intellectual disability, epilepsy, language impairments and autistic features. Associated SNPs and heterozygous deletions in CNTNAP2 were subsequently reported in autism, schizophrenia and other psychiatric or neurological disorders. We aimed to comprehensively examine evidence for the role of CNTNAP2 in susceptibility to psychiatric disorders, by the analysis of multiple classes of genetic variation in large genomic datasets. In this study we used: i) summary statistics from the Psychiatric Genomics Consortium (PGC) GWAS for seven psychiatric disorders; ii) examined all reported CNTNAP2 structural variants in patients and controls; iii) performed cross-disorder analysis of functional or previously associated SNPs; and iv) conducted burden tests for pathogenic rare variants using sequencing data (4,483 ASD and 6,135 schizophrenia cases, and 13,042 controls). The distribution of CNVs across CNTNAP2 in psychiatric cases from previous reports was no different from controls of the database of genomic variants. Gene-based association testing did not implicate common variants in autism, schizophrenia or other psychiatric phenotypes. The association of proposed functional SNPs rs7794745 and rs2710102, reported to influence brain connectivity, was not replicated; nor did predicted functional SNPs yield significant results in meta-analysis across psychiatric disorders at either SNP-level or gene-level. Disrupting CNTNAP2 rare variant burden was not higher in autism or schizophrenia compared to controls. Finally, in a CNV microarray study of an extended bipolar disorder family with 5 affected relatives we previously identified a 131kb deletion in CNTNAP2 intron 1, removing a FOXP2 transcription factor binding site. Quantitative-PCR validation and segregation analysis of this CNV revealed imperfect segregation with BD.

This large comprehensive study indicates that CNTNAP2 may not be a robust risk gene for psychiatric phenotypes.
Genetic mutations that disrupt both copies of the \textit{CNTNAP2} gene lead to severe disease, characterized by profound intellectual disability, epilepsy, language difficulties and autistic traits, leading to the hypothesis that this gene may also be involved in autism given some overlapping clinical features with this disease. Indeed, several large DNA deletions affecting one of the two copies of \textit{CNTNAP2} were found in some patients with autism, and later also in patients with schizophrenia, bipolar disorder, ADHD and epilepsy, suggesting that this gene was implicated in several psychiatric or neurologic diseases. Other studies considered genetic sequence variations that are common in the general population, and suggested that two such sequence variations in \textit{CNTNAP2} predispose to psychiatric diseases by influencing the functionality and connectivity of the brain. To better understand the involvement of \textit{CNTNAP2} in risk of mental illness, we performed several genetic analyses using a series of large publicly available or in-house datasets, comprising many thousands of patients and controls. Furthermore, we report the deletion of one copy of \textit{CNTNAP2} in two patients with bipolar disorder and one unaffected relative from an extended family where five relatives were affected with this condition. Despite the previous consideration of \textit{CNTNAP2} as a strong candidate gene for autism or schizophrenia, we show little evidence across multiple classes of DNA variation, that \textit{CNTNAP2} is likely to play a major role in risk of psychiatric diseases.

\textbf{Introduction}

The contactin-associated protein-like 2 (\textit{CNTNAP2}) is located on chromosome 7q35-36.1, and consists of 24 exons spanning 2.3Mb, making it one of the largest protein coding genes in the human genome. This gene encodes the CASPR2 protein, related to the neurexin superfamily, which localises with potassium channels at the juxtaparanodal regions of the Ravier nodes in myelinated axons, playing a crucial role in the clustering of potassium channels required for conduction of axon potentials [1]. \textit{CNTNAP2} is expressed in the spinal cord, prefrontal and frontal cortex, striatum, thalamus and amygdala; this pattern of expression is preserved throughout the development and adulthood [2, 3]. Its function is related to neuronal migration, dendritic arborisation and synaptic transmission [4]. The crucial role of \textit{CNTNAP2} in the human brain became clear in 2006 when Strauss et al, reported homozygous mutations in Old Order Amish families segregating with a severe Mendelian condition, described as cortical dysplasia-focal epilepsy (CDFE) syndrome (OMIM 610042) [5]. In 2009, additional patients with recessive mutations in \textit{CNTNAP2} were reported, with clinical features resembling Pitt-Hopkins syndrome [6]. To date 33 patients, mostly from consanguineous families, have been reported with homozygous or compound deletions and truncating mutations in \textit{CNTNAP2} [5–9], and are collectively described as having CASPR2 deficiency disorder [7]. The common clinical features in this phenotype include severe intellectual disability (ID), seizures with age of onset at two years and concomitant speech impairments or language regression. The phenotype is often accompanied by dysmorphic features, autistic traits, psychomotor delay and focal cortical dysplasia.

\textit{CNTNAP2} is also thought to contribute to diverse phenotypes in patients with interstitial or terminal deletions at 7q35 and 7q36. Interstitial or terminal deletions encompassing \textit{CNTNAP2} and several other genes have been described in individuals with ID, seizures, craniofacial anomalies, including microcephaly, short stature and absence of language [10]. The severe language impairments observed in patients with homozygous mutations or karyotypic abnormalities involving \textit{CNTNAP2} suggested a possible functional interaction with \textit{FOXP2}, a

\textbf{Author summary}

Genetic mutations that disrupt both copies of the \textit{CNTNAP2} gene lead to severe disease, characterized by profound intellectual disability, epilepsy, language difficulties and autistic traits, leading to the hypothesis that this gene may also be involved in autism given some overlapping clinical features with this disease. Indeed, several large DNA deletions affecting one of the two copies of \textit{CNTNAP2} were found in some patients with autism, and later also in patients with schizophrenia, bipolar disorder, ADHD and epilepsy, suggesting that this gene was implicated in several psychiatric or neurologic diseases. Other studies considered genetic sequence variations that are common in the general population, and suggested that two such sequence variations in \textit{CNTNAP2} predispose to psychiatric diseases by influencing the functionality and connectivity of the brain. To better understand the involvement of \textit{CNTNAP2} in risk of mental illness, we performed several genetic analyses using a series of large publicly available or in-house datasets, comprising many thousands of patients and controls. Furthermore, we report the deletion of one copy of \textit{CNTNAP2} in two patients with bipolar disorder and one unaffected relative from an extended family where five relatives were affected with this condition. Despite the previous consideration of \textit{CNTNAP2} as a strong candidate gene for autism or schizophrenia, we show little evidence across multiple classes of DNA variation, that \textit{CNTNAP2} is likely to play a major role in risk of psychiatric diseases.
gene for which heterozygous mutations lead to a monogenic form of language disorder [11]. Interestingly, Vernes et al., found that the FOXP2 transcription factor has a binding site in intron 1 of CNTNAP2, regulating its expression [12]. Considering that a large proportion of autistic patients show language impairments and most individuals with homozygous mutations in CNTNAP2 manifest autistic features, several studies investigated the potential involvement of CNTNAP2 in autism spectrum disorder (ASD). In particular, two pioneering studies showed that single nucleotide polymorphism (SNP) markers rs2710102 and rs7794745 were associated with risk of ASD [13, 14]. These studies were the first implicating CNTNAP2 in autism, and opened a chapter of additional analyses in ASD and other psychiatric phenotypes during the next decade. In subsequent studies, rs2710102 was implicated in early language acquisition in the general population [15], and showed functional effects on brain activation in neuroimaging studies [16–19]. Furthermore, genotypes at rs7794745 were associated with reduced grey matter volume in the left superior occipital gyrus in two independent studies [20, 21], and alleles of this SNP were reported to affect voice-specific brain function [22]. Genetic associations with ASD for these, and several other SNPs in CNTNAP2, have been reported in a number of studies [23–28]. Along with the first reports of SNPs associated with ASD, copy number variant (CNV) deletions have also been described in ID or ASD patients, which were proposed to be highly penetrant disease-causative mutations [13, 29–38]. To better understand the role of CNTNAP2 in ASD pathophysiology, knockout mice were generated. Studies of these mice reported several neuronal defects when both copies of CNTNAP2 are mutated: abnormal neuronal migration, reduction of GABAergic interneurons, deficiency in excitatory neurotransmission, and the delay of myelination in the neocortex [2, 39, 40].

These intriguing findings prompted additional investigations of CNTNAP2 across other psychiatric disorders or language-related traits, with additional reports of SNPs being associated with schizophrenia (SCZ), bipolar disorder (BD), specific language impairment (SLI) and several other phenotypes or traits [12, 15, 41–50]. Consequently, other studies reported CNV deletions in CNTNAP2 in schizophrenia [51, 52], bipolar disorder [52–54], and ADHD [55]; but also in neurological disorders, especially epilepsy [56–61], and language-related phenotypes [62–65]. Interestingly, several of these structural variants were found in intron 1 of CNTNAP2, encompassing the FOXP2 transcription factor binding site. Epilepsy is clinically frequent in psychiatric disorders, especially schizophrenia and bipolar disorder [66–69], and is present in approximately 20% of autistic patients [70, 71]. Similarly, cognitive deficits involving language-related domains are also comorbid traits in schizophrenia and bipolar disorder [67, 72–74], and are common clinical features in ASD [75], with many ASD patients remaining non-verbal throughout life [75, 76].

While CNTNAP2 is now considered a strong candidate gene for ASD and psychiatric disease more generally (summarised in Table 1), several of these early supportive studies were performed with limited sample sizes, or were individual case reports which lacked comparison with control individuals, hence providing circumstantial evidence as a psychiatric risk gene. We therefore aimed in this current study to perform systematic genetic analyses with large datasets to examine the evidence for a role of the CNTNAP2 gene in multiple psychiatric phenotypes—performing a comprehensive analysis of common and rare variants, CNVs and de novo mutations—using both publicly available datasets and in-house data.

Results

Analysis of CNTNAP2 common single nucleotide variation in the susceptibility of psychiatric disorders

During the last decade, several association studies have been performed to assess the role of common variants of CNTNAP2 in autism or speech-related phenotypes [12–15, 23–28, 46–48,
50], as well as several other psychiatric phenotypes [41–45, 49]. In Table 2, we summarise all markers found significantly associated in these previous studies, and report the corresponding P-value from the Psychiatric Genomics Consortium GWAS for seven major psychiatric disorders: ADHD, anorexia nervosa, ASD, bipolar disorder, MDD, OCD and schizophrenia. Nominal associations were found with ASD for the following markers: rs802524 ($P = 0.016$), rs802568 ($P = 0.008$), rs17170073 ($P = 0.008$), and rs2710102 ($P = 0.036$; which is highly correlated with 4 SNPs: rs759178, rs1922892, rs2538991, rs2538976). Furthermore, nominal association was also observed with schizophrenia for rs1859547 ($P = 0.044$); with ADHD for rs1718101 ($P = 0.038$); with MDD for rs12670868 ($P = 0.047$), rs1922892, rs2538991, rs2538976). Furthermore, nominal association was also observed with schizophrenia for rs1859547 ($P = 0.044$); with ADHD for rs1718101 ($P = 0.038$); with MDD for rs12670868 ($P = 0.047$), rs17236239 ($P = 0.006$), rs4431523 ($P = 0.001$); and with anorexia nervosa for rs700273 ($P = 0.013$). The nominal association with ASD at rs1770073 and rs2710102 represents the only case in which the association in the original report replicates in the PGC dataset for the same phenotype. The two SNPs rs7794745 and rs2710102, which were repeatedly reported as being associated in earlier studies with smaller sample size and proposed to be functional SNPs, were not strongly associated with any phenotype (the most significant signal being $P = 0.036$ for rs2710102 in ASD). None of those associations survived corrections for multiple comparisons (Table 2).

### Gene-based analysis of cross-disorder associations

Next, we explored the contribution of common variants across CNTNAP2 by performing a gene-based association study in MAGMA using GWAS summary statistics from PGC data of seven psychiatric disorders in European populations (Table 3). Association plots for all SNPs included in analysis of each individual phenotype are shown in supporting information (S1 Fig).

The test included a dense coverage of SNPs across CNTNAP2: from 1,214 SNPs in MDD up to 12,264 SNPs in schizophrenia. The results suggest that common variants overall do not contribute to disease susceptibility of these phenotypes (Table 3). The most significant association observed was for MDD phase 1 analysis ($P = 0.029$), which is the dataset with the most modest coverage of markers.

To explore whether any gene-based signal is not being detected due to a high signal-to-noise ratio (i.e. inclusion of a large number of SNPs of no functional consequence), we selected
63 predicted functional SNPs in CNTNAP2 and performed a meta-analysis across psychiatric disorders (for regional association plot, see S2 Fig). Nominal significance of association was observed for 11 predicted functional SNPs with $P$-values ranging from 0.01 and 0.05, but none survive correction for multiple comparisons (Table 4).

The only predicted functional SNP which was previously reported as being associated with ASD was rs34712024 [25], but this variant was not associated with autism in the PGC dataset ($P = 0.67$; Table 2), nor other psychiatric phenotypes examined separately or together (Tables 2–4). MAGMA gene-based association analysis using this more restricted pool of common putative functional variants revealed significant association with ADHD after correction for multiple testing (corrected $P$-value = 0.033) and a nominal association with schizophrenia.

### Table 2. Common SNPs in CNTNAP2 previously reported to be associated in psychiatric diseases, and their evidence for association in PGC datasets.

| SNP          | Location | Disease (Ref) | ASD   | SCZ   | BD    | ADHD | MDD  | AN   | OCD  |
|--------------|----------|---------------|-------|-------|-------|------|------|------|------|
| rs34712024   | Promoter | ASD [25]      | 0.672^ | 0.45  | 0.099 | 0.442| N/A  | 0.283| 0.295|
| rs802524     | Intron 1 | SCZ, BD [41]  | 0.016^ | 0.081 | 0.058 | 0.210| 0.070| 0.143| 0.039|
| rs802568     | Intron 1 | SCZ, BD [41]  | 0.008^ | 0.061 | 0.312 | 0.047| 0.054| 0.321| 0.279|
| rs1710073    | Intron 1 | ASD [26]      | 0.008 | 0.903 | 0.558 | 0.883| 0.306| 0.031| 0.101|
| rs1718101    | Intron 1 | ASD [27]      | 0.076^ | 0.257 | 0.215 | 0.038| 0.255| 0.243| 0.029|
| rs700273     | Intron 1 | ALD [42]      | 0.840 | 0.655 | 0.837 | 0.544| 0.338| 0.013| 0.554|
| rs7794745    | Intron 2 | ASD [14, 23, 24]| 0.906 | 0.734 | 0.498 | 0.393| 0.173| 0.877| 0.503|
| rs10251794   | Intron 3 | OPN [43]      | 0.301 | 0.365 | 0.155 | 0.452| 0.047| 0.255| 0.243| 0.029|
| rs7804520    | Intron 3 | ASD [28]      | 0.378 | 0.277 | 0.236 | 0.155| 0.568| 0.506| 0.682|
| rs1603450    | Intron 8 | LAN [15]      | 0.445 | 0.166 | 0.643 | 0.141| 0.010| 0.951| 0.577|
| rs826824     | Intron 9 | MDD (male only) [44]| 0.218 | 0.181 | 0.256 | 0.317| 0.266| 0.736| 0.199|
| rs1859547    | Intron 11| SCZ [45]      | 0.697 | 0.044 | 0.431 | 0.225| 0.939| 0.729| 0.154|
| rs851715^   | Intron 13| SLI [12]      | 0.448 | 0.496 | 0.572 | 0.067| 0.601| 0.920| 0.411|
| rs10246256^ | Intron 13| SLI [12, 46]| 0.429 | 0.613 | 0.508 | 0.070| 0.601| 0.851| 0.454|
| rs2710102*  | Intron 13| ASD, SLI, DYS, SM, ANX, LAN, MDD [12, 13, 15, 23, 46–49]| 0.036 | 0.893 | 0.801 | 0.911| 0.346| 0.383| 0.351|
| rs759178#   | Intron 13| SLI, LAN [12, 15]| 0.037 | 0.890 | 0.799 | 0.929| 0.332| 0.363| 0.347|
| rs1922892^  | Intron 13| SLI [12]      | 0.039 | 0.908 | 0.794 | 0.940| 0.332| 0.359| 0.346|
| rs2538991^  | Intron 13| SLI [12]      | 0.041 | 0.852 | 0.797 | 0.989| 0.332| 0.359| 0.366| 0.338|
| rs17236239  | Intron 13| ASD, SCZ, SLI [12, 23, 27, 46, 49]| 0.142 | 0.290 | 0.278 | 0.883| 0.006| 0.622| 0.954|
| rs2538976*  | Intron 13| SLI, SSD [12, 50]| 0.051 | 0.718 | 0.692 | 0.812| 0.358| 0.408| 0.424|
| rs2215798   | Intron 13| ASD [26]      | 0.5   | 0.469 | 0.361 | 0.742| 0.030| 0.568| 0.281|
| rs4431523   | Intron 13| SLI [12]      | 0.275 | 0.844 | 0.676 | 0.614| 0.001| 0.933| 0.972|
| rs2710117   | Intron 14| SLI, MDD [12, 46, 49]| 0.1477| 0.6701| 0.7566| 0.2106| 0.894| 0.2710121| 0.993| 0.321|
| rs2710093   | Intron 14| ASD [26]      | 0.4077| 0.2891| 0.02819| 0.2943| 0.090| 0.2710091| 0.8416| 0.2767|

The disease for which association at each listed SNP is given, along with the reference number for each study and the approximate location of each variant within the CNTNAP2 gene structure. On the right, the $P$-value from each Psychiatric Genomics Consortium (PGC) dataset is reported. Where the associated SNP was not found in the GWAS summary statistic data, results for an alternative SNP are shown in parenthesis ($r^2 = 1$). Putative functional SNPs rs7794745 and rs2710102 are underlined. No association survives correction for multiple independent tests ($P < 3.8E-04$), but $P$-values < 0.05 are shown in bold. Abbreviations: ASD, autism spectrum disorder; SLI, specific language impairment; DYS, dyslexia; ANX, social anxiety; LAN, language in general population; SCZ, schizophrenia; BD, bipolar disorder; ALD, Alcohol dependence; OPN, Openness general population; MDD, major depressive disorder; SSD, speech sound disorder; N/A, SNP not genotyped & $r^2$ > 0.97 across the following SNPs: rs851715 and rs10246256

#, summary data at this SNP was not included in the latest autism GWAS (PGC2) but was present in the previous data set which included 5,305 ASD cases and 5,305 controls.

https://doi.org/10.1371/journal.pgen.1007535.t002
De novo variants in CNTNAP2

De novo variants in protein-coding genes which are predicted to be functionally damaging are considered to be highly pathogenic and have been extensively explored to implicate genes in psychiatric diseases, especially in ASD and schizophrenia [77]. We explored publicly available sequence data from previous projects in psychiatric disorders to assess the rate of coding de novo variants in CNTNAP2 using two databases (NPdenovo, http://www.wzgenomics.cn/NPdenovo/; and denovo-db, http://denovo-db.gs.washington.edu/denovo-db/). No truncating or missense variants were identified across CNTNAP2 in 15,539 families (including 2,163 controls), and synonymous variants were reported in only two probands with developmental disorder (Table 5).

Pathogenic Ultra-Rare Variants (URV) of CNTNAP2 in ASD and Schizophrenia

Finally, we explored the potential impact of pathogenic ultra-rare variants (URV) in CNTNAP2 using available sequencing datasets of 4,483 patients with ASD and 6,135 patients with schizophrenia compared with 13,042 controls. We considered only those variants predicted to be pathogenic in both SIFT and Polyphen and which are ultra-rare (MAF < 0.0001 in Non-Finnish European population; S3 Table). No difference in the total number of URV was observed between controls and patients with ASD (P = 0.11), or schizophrenia (P = 0.78) (Table 6).

Structural variants affecting CNTNAP2 amongst psychiatric phenotypes

Several deletions and duplications have been described in neuropsychiatric phenotypes thus far. In Fig 1, we present a comprehensive representation of all previously reported structural variants found in CNTNAP2 in psychiatric disorders such as ASD or ID [13, 29–38], schizophrenia or bipolar disorder [51–54, 78], ADHD [55], neurologic disorders such as epilepsy, Tourette syndrome or Charcot-Marie-Tooth [56–61]; and finally language-related phenotypes such as speech delay, childhood apraxia of speech and dyslexia [62–65]. Interestingly, the
Table 4. Cross psychiatric disorders meta-analysis of 63 predicted functional SNPs.

| SNPs       | Allele | Function | Datasets                  | I     | P-val | OR  |
|------------|--------|----------|---------------------------|-------|-------|-----|
| rs17480644 | A/G    | TFBS     | ADHD, AN, BD, OCD, SCZ    | 8     | 0.083 | 1.039 |
| rs1260124  | A/T    | TFBS     | ADHD, AN, ASD, BD, OCD, SCZ | 0   | 0.635 | 0.996 |
| rs35796336 | T/C    | TFBS     | AN, ASD, BD, OCD, SCZ     | 0     | **0.049** | 1.027 |
| rs10277276 | T/C    | TFBS     | BD, OCD, SCZ              | 0     | 0.764 | 0.992 |
| rs34712024 | A/G    | TFBS     | ADHD, AN, BD, OCD, SCZ    | 32    | 0.626 | 1.012 |
| rs2462603  | A/G    | TFBS     | ADHD, AN, ASD, BD, OCD, SCZ | 0   | 0.303 | 0.993 |
| rs1639484  | A/T    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ | 0   | 0.376 | 1.005 |
| rs12703814 | A/G    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ | 0   | 0.965 | 0.999 |
| rs1639447  | A/C    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ | 0   | 0.376 | 0.990 |
| rs769344   | C/G    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ | 0   | 0.778 | 0.996 |
| rs10280967 | A/G    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ | 0   | 0.639 | 0.996 |
| rs10243142 | T/C    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ | 0   | 0.738 | 1.002 |
| rs12535047 | T/C    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ, MDD | 0 | 0.327 | 0.993 |
| rs347201   | A/G    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ | 0   | 0.089 | 1.011 |
| rs13234249 | T/C    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ, MDD | 2 | 0.101 | 1.011 |
| rs1266908  | T/C    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ | 0   | 0.914 | 0.999 |
| rs11972428 | T/G    | SeqCons  | ADHD, AN, BD, OCD, SCZ    | 0     | 0.672 | 1.012 |
| rs34222835 | A/G    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ | 4     | **0.045** | 0.979 |
| rs10261412 | A/G    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ, MDD | 52   | 0.808 | 0.995 |
| rs1826843  | A/G    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ, MDD | 20  | 0.153 | 0.990 |
| rs1710356  | A/G    | SeqCons  | ADHD, AN, BD, OCD, SCZ    | 0     | 0.137 | 0.972 |
| rs4726831  | A/C    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ | 18    | 0.294 | 0.990 |
| rs10279700 | T/C    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ, MDD | 26   | 0.857 | 0.998 |
| rs35701811 | A/G    | SeqCons  | ADHD, AN, BD, OCD, SCZ    | 0     | 0.899 | 0.998 |
| rs899617   | T/C    | SeqCons  | ADHD, AN, BD, OCD, SCZ    | 0     | **0.026** | 1.014 |
| rs747140   | C/G    | SeqCons  | ADHD, AN, BD, OCD, SCZ    | 0     | **0.025** | 1.014 |
| rs779087   | A/G    | SeqCons  | ADHD, AN, BD, OCD, SCZ    | 0     | 0.091 | 1.011 |
| rs3459169  | A/G    | Splicing | ADHD, AN, BD, OCD, SCZ    | 15    | 0.067 | 1.014 |
| rs6970064  | A/G    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ, MDD | 7 | 0.462 | 1.004 |
| rs1710640  | A/G    | SeqCons  | ADHD, AN, BD, OCD, SCZ    | 0     | **0.018** | 1.041 |
| rs16883690 | A/C    | SeqCons  | BD, SCZ                   | 66    | 0.673 | 1.066 |
| rs7797724  | T/C    | SeqCons  | BD, SCZ                   | 0     | **0.047** | 1.592 |
| rs851659   | A/C    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ | 9     | 0.349 | 0.993 |
| rs35815165 | /AA    | SeqCons  | ADHD, AN                   | 0     | 0.831 | 1.002 |
| rs13247212 | T/C    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ | 0     | 0.797 | 0.996 |
| rs1177007  | A/G    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ, MDD | 0 | 0.238 | 1.008 |
| rs12154883 | T/G    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ | 0     | 0.645 | 1.006 |
| rs13438769 | T/C    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ, MDD | 0 | 0.057 | 1.018 |
| rs2707580  | T/G    | SeqCons  | ADHD, AN, BD, OCD, SCZ    | 0     | 0.146 | 0.990 |
| rs2707581  | T/C    | SeqCons  | ADHD, AN, BD, OCD, SCZ    | 0     | 0.156 | 0.990 |
| rs2141955  | A/G    | SeqCons  | ADHD, AN, BD, OCD, SCZ    | 0     | **0.032** | 1.015 |
| rs34347668 | A/C    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ | 0     | 0.973 | 1.000 |
| rs4725756  | A/C    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ, MDD | 0 | **0.015** | 0.984 |
| rs2888540  | T/C    | SeqCons  | ADHD, AN, ASD, BD, OCD, SCZ, MDD | 0 | **0.023** | 1.014 |
| rs1710789  | A/T    | SeqCons  | ADHD, AN, BD, OCD, SCZ    | 0     | 0.566 | 1.010 |
| rs1710801  | A/C    | SeqCons  | ADHD, AN, BD, OCD, SCZ    | 0     | 0.643 | 0.989 |
| rs10279343 | T/C    | SeqCons  | ADHD, AN, BD, OCD, SCZ    | 1     | 0.568 | 1.014 |

(Continued)
reported structural variants frequently map in intron 1, and extend to exon 4 in some cases. The distribution of those structural variants across different phenotypes does not vary with those found in control populations from the database of genomic variants (http://dgv.tcag.ca/dgv/app/home) (Fig 1), suggesting that structural variants in CNTNAP2 are not rare events associated exclusively to disease but are present with rare frequency in the general population. Unfortunately, as many reported CNVs come from individual case reports for which the number of subjects screened is not reported, direct frequency comparisons of this data are not meaningful.

Examination of an intronic deletion in CNTNAP2 in an extended family with bipolar disorder

CNV microarray analysis was previously performed in two affected individuals from an extended family which included five relatives affected with bipolar I disorder [78]. A drop in

Table 4. (Continued)

| SNPs      | Allele | Function        | Datasets                      | I  | P-val | OR  |
|-----------|--------|-----------------|-------------------------------|----|-------|-----|
| rs1122622 | A/C    | SeqCons         | AN, BD, OCD, SCZ              | 66 | 0.745 | 1.026|
| rs5888312 | -/A    | SeqCons         | ADHD, AN                      | 70 | 0.744 | 1.010|
| rs9648691 | A/G    | SeqCons, Splicing| ADHD, AN, ASD, BD, OCD, SCZ, MDD | 64 | 0.806 | 1.002|
| rs987456  | A/C    | miRNA           | ADHD, AN, ASD, BD, OCD, SCZ, MDD | 29 | 0.570 | 1.004|
| rs2717809 | C/G    | miRNA           | AN, BD, OCD, SCZ              | 0  | 0.746 | 0.989|
| rs2530312 | A/G    | miRNA           | ADHD, AN, ASD, BD, OCD, SCZ   | 70 | 0.485 | 0.990|
| rs3194    | A/C    | miRNA           | ADHD, AN, ASD, BD, OCD, SCZ, MDD | 68 | 0.797 | 1.003|
| rs10243309| C/T    | miRNA           | AN, MDD                       | 15 | 0.125 | 1.149|
| rs1710999 | A/G    | miRNA           | AN, BD, OCD, SCZ, MDD         | 0  | 0.026 | 0.917|
| rs2530311 | A/G    | miRNA           | ADHD, AN, ASD, BD, OCD, SCZ, MDD | 66 | 0.975 | 0.999|
| rs1717000 | T/C    | miRNA           | ADHD, AN, BD, OCD, SCZ, MDD   | 0  | 0.583 | 0.988|
| rs10251347| C/G    | miRNA           | ADHD, AN, BD, OCD, SCZ, MDD   | 0  | 0.428 | 0.986|
| rs2717829 | C/G    | miRNA           | ADHD, AN, BD, OCD, SCZ, MDD   | 50 | 0.820 | 0.997|
| rs1028038 | A/G    | miRNA           | AN, BD, OCD, SCZ, MDD         | 0  | 0.031 | 1.087|
| rs2530310 | T/C    | miRNA           | ADHD, AN, ASD, BD, OCD, SCZ, MDD | 67 | 0.666 | 0.994|
| rs1717006 | T/C    | miRNA           | ADHD, AN, BD, OCD, SCZ        | 0  | 0.493 | 0.985|

For each predicted functional SNP, the alternative alleles and predicted function are listed. P-values (P-val) were calculated considering fixed-model effect, except SNPs with evidence of heterogeneity (I^2 > 50) where odds ratios (OR) were considered under random-effects. Nominally significant associations are indicated in bold (P-values < 0.05), but none exceed correction for multiple testing (P<7.9E-04). Abbreviations: TFBS, transcription factor binding site; SeqCons, sequence conserved nucleotide across species; miRNA, predicted miRNA binding site; Splicing, exonic splicing enhancer (ESE).

https://doi.org/10.1371/journal.pgen.1007535.t004

Table 5. CNTNAP2 de novo variants identified across several disease-specific sequencing projects.

| Phenotype | N Families | Intrinsic | Synonymous | Missense |
|-----------|------------|-----------|------------|----------|
| ASD       | 6,171      | 106       | -          | -        |
| SCZ       | 1,164      | -         | -          | -        |
| EE        | 647        | -         | -          | -        |
| ID        | 1,101      | -         | -          | -        |
| DD        | 4,293      | -         | 2          | -        |
| Controls  | 2,163      | 13        | -          | -        |

The number (N) of families in each dataset examined is given. The full list of de novo variants observed is listed in S2 Table. Abbreviations: ASD, autism spectrum disorder; SCZ, schizophrenia; EE, epilepsy; ID, intellectual disability; DD, developmental disability.

https://doi.org/10.1371/journal.pgen.1007535.t005
signal intensity for 340 consecutive probes was compatible with a deletion of 131 kb in intron 1 of CNTNAP2 (hg19/chr7:146203548–146334635; Fig 2A), encompassing the described binding site for the transcription factor FOXP2 (hg19/chr7:146215016–146215040) [12]. The deletion was detected in one of the two affected individuals examined by CNV array. To infer deletion segregation amongst additional relatives, WES-derived genotypes were used to create haplotypes across chromosome 7q35 (Fig 2B). CNV segregation (by haplotype inference) was uninformative due to: 1) incomplete genotype data (unaffected descendants of deceased patient 8404 were not included in the WES study) and 2) a likely recombination at 7q35 in the family. Thus experimental validation and CNV genotyping was performed in all individuals with DNA available to assess the presence of the CNTNAP2 intronic deletion and its disease association. Using quantitative PCR, the deletion was validated in proband subject 8401, and was detected in one unaffected descendant of deceased patient 8404 (Fig 2B and 2C), implying

Table 6. Burden analysis of CNTNAP2 ultra-rare variants (URVs) in ASD and SCZ.

|                | N Individuals | N Pathogenic URVs | P-Value |
|----------------|---------------|-------------------|---------|
| Controls       | 13,042        | 59                |         |
| SCZ            | 6,135         | 26                | 0.78    |
| ASD            | 4,483         | 29                | 0.11    |

The selection of variants included missense variants which are predicted to be pathogenic, truncating variants and canonical splice-site variants. The full list of URVs observed is provided in S3 Table. Abbreviations: SCZ, schizophrenia; ASD, autism spectrum disorder.

https://doi.org/10.1371/journal.pgen.1007535.t006

Fig 1. Overview of heterozygous CNVs spanning the CNTNAP2 gene across several diseases. Abbreviations: ID (Intellectual disability), ASD (autism spectrum disorder), SCZ (schizophrenia), BD (bipolar disorder), ADHD (Attention-deficit/hyperactivity disorder), EP (epilepsy), TS (Tourette syndrome), CMT2 (axonal Charcot-Marie-Tooth), and SS (Speech spectrum: speech delay, childhood apraxia of speech and dyslexia). In parenthesis is reported the reference to each study. *, additional rearrangements reported in this patient. The dashed lines represent the exons and the upper box shows the position of the FOXP2 binding site. In dark shading, CNVs >80kb found in the general populations from the Database of Genomic Variants are shown.

https://doi.org/10.1371/journal.pgen.1007535.g001
that: 1) affected subject 8404 would have carried the deletion, had DNA been available; and 2) the CNV is unlikely to be highly penetrant as it was observed in an unaffected adult relative. The structural variant was not detected in the remaining affected relatives and therefore did not segregate with disease status in this family (Fig 2B).

**Discussion**

During the last decade, the CNTNAP2 gene has received considerable attention in the psychiatric genetics field, with a number of studies examining gene dosage, and common or rare variants associations across multiple major psychiatric disorders, which together provided compelling evidence that CNTNAP2 may be a risk gene with pleiotropic effects in psychiatry. While homozygous mutations in this gene lead to a rare and severe condition described as CASPR2 deficiency disorder (CDD) [7], characterized by profound intellectual disability, epilepsy, language impairment or regression [7, 8], heterozygous mutations or common variants have been suggested to be implicated in autism, whose clinical features overlap with some
observed in CDD. CNTNAP2 is categorised in the SFARI database as syndromic gene and one of the highest-ranking "strong candidate" gene for ASD (https://gene.sfari.org). Heterozygous deletions encompassing the CNTNAP2 gene were described not only in autism but a wide range of phenotypes, including psychiatric or neurologic disorders, and language-related deficiencies. These structural variants were generally described as causative or highly penetrant [13, 29, 31, 55, 57, 59].

Examination of the distribution of all structural variants described thus far in psychiatric or neurologic patients showed comparable localisation to those found in the general population, suggesting that structural variants affecting CNTNAP2 may be less relevant in disease susceptibility than previously considered. We were not able to directly compare frequencies of observed structural variants in cases versus controls due to reporting bias in case reports and a lack of information on how many cases were screened to identify those subjects with reportable CNTNAP2 CNVs, which is a limitation of this study. In the ExAC database, CNTNAP2 had fewer CNV variants than expected (11 observed vs. 16 expected, z = 0.43; http://exac.broadinstitute.org), and its haploinsufficiency score of 0.59 is in the 8th decile of all genes [79], suggesting that CNTNAP2 has a tendency to be intolerant to structural variants. A specific case-control CNV analysis is needed to examine CNV frequency differences, but would require a very large sample due to the rarity of CNVs at this locus. A close clinical psychiatric examination of the 66 parents with heterozygous deletions across CNTNAP2 of CDD provides information on the prevalence of psychiatric conditions in individuals carrying CNTNAP2 CNVs. All heterozygous family members carrying deletions or truncating mutations were described as phenotypically healthy, suggesting a lack of correlation between these deletions and any major psychiatric condition. Furthermore, parents who were carriers for heterozygous deletions in psychiatric/neurologic patients were described as unaffected at the time of reporting [13, 29, 31, 37, 54, 62], with two exception: one father of a proband with neonatal convulsion, and another father of an epileptic patient, were reported as affected [56, 59]. Moreover, discordant segregation for deletions in CNTNAP2 was also observed in an ASD sib-pair [13]. Several psychiatric patients who were reported to carry heterozygous structural variants in CNTNAP2 were also described with translocations or other chromosomal abnormalities [29, 30, 33, 34, 56, 58, 62–65], therefore it is possible that these aberrations may explain the phenotype independently from the observed CNVs in CNTNAP2.

We also describe a new CNV deletion which does not segregate with disease in an extended family with bipolar disorder. This CNV removes the FOXP2 transcription factor binding site in intron 1 of CNTNAP2, and overlaps with structural variants described in a number of other psychiatric patients. This heterozygous deletion was identified in two individuals with bipolar I disorder from an extended family with five affected members, but was observed also in one unaffected relative (who underwent diagnostic interview at age >40 and therefore was beyond the typical age of symptom onset). Hence, the deletion was not segregating with the disease and is unlikely to represent a highly penetrant risk variant in this family, although we cannot exclude a multiple hit model where the CNV deletion interacts with other etiologic risk variants at other loci to exert phenotypic effect.

CNTNAP2−/− knock-out mice have been proposed as valid animal model for ASD considering the phenotypic similarities between ASD and the CASPR2 deficiency disorder [2]. CNTNAP2−/− knock-out mice showed abnormalities in the arborisation of dendrites, maturation of dendritic spines, defects in migration of cortical projection neurons, and reduction of GABAergic interneurons [2, 4]. Controversially, ASD is not a core feature in the most recent patient series reported with CASPR2 deficiency disorder [7, 8]. The association previously proposed around the relationship between heterozygous deletions in CNTNAP2 and ASD does not have a support from mouse models, as heterozygous mice did not show any behavioural or
neuropathological abnormalities that were observed in homozygous knockouts [2]. Notwithstanding this, it is possible that the combination of heterozygous CNTNAP2 deletions in a genomic background of increased risk (through inheritance of other common and rare risk variants at other loci) may lead to psychiatric, behavioural or neuropathological abnormalities.

Common variants in CNTNAP2 are another class of genetic variation associated with several psychiatric or language-related phenotypes. The most interesting finding from studies of this variant class converge on markers rs7794745 and rs2710102, originally reported in ASD [13, 14], and replicated later in ASD or implicated in other phenotypes [12, 15, 23, 24, 46–48]. Neuroimaging studies have supported the notion that these common variants play a role in psychiatric disorders. SNP rs2710102 has been implicated in brain connectivity in healthy individuals [16, 18, 19], and rs7794745 was implicated in audio-visual speech perception [80], voice-specific brain function [22], and was associated with reduced grey matter volume in left superior occipital gyrus [20, 21]. These studies focused principally on language tasks in general population, given the reported suggestive implications of CNTNAP2 in language impairment traits of ASD or language-related phenotypes. However, the direct role of CNTNAP2 in language is still unclear; indeed the language regression observed in patients with CASPR2 deficiency disorder are concomitant with seizure onset and may represent a secondary phenotypic effect caused by seizures [7]. On the other hand, the first genetic association of rs7794745 and rs2710102 with ASD, as well as the other psychiatric diseases were based in studies with limited sample size, and recent studies failed to replicate associations between the two markers and ASD [81, 82]. Individual alleles associated in the past with limited numbers of patients warrant replications in adequately powered samples to ascertain bona fide findings considering the small size effects of common variants [83], such as that attempted here. Using the largest case-control cohorts currently publicly available (PGC datasets), we did not find evidence for significant association of previously reported common variants, or a combined effect for common variants of CNTNAP2 in the susceptibility of psychiatric disorders, nor did we find predicted functional SNPs with a role across disorders.

Finally, we examine evidence for rare variant contributions in CNTNAP2. Rare variants in the promoter or coding region were reported to play a role in the pathophysiology of ASD [25, 33], although a recent study including a large number of cases and controls did not find association of rare variants of CNTNAP2 in ASD [84]. Here we report the largest sample investigated thus far in ASD and schizophrenia, which suggests that rare variants in CNTNAP2 do not play a major role in these two psychiatric disorders. Furthermore, examination of de novo variants in combined psychiatric sequencing projects of over 15,500 trios suggest that de novo variants in CNTNAP2 do not increase risk for psychiatric disorders.

While functional studies show a relationship between certain deletions or rare variants of CNTNAP2 with neuronal phenotypes relevant to psychiatric illness [25, 54, 85], we show that the genetic link between these variants and psychiatric phenotypes is tenuous. However, this does not dispel the evidence that the CNTNAP2 gene, or specific genetic variations within this gene, may have a real impact on neuronal functions or variability of brain connectivity in the general population.

It is now possible to combine large datasets to ascertain the real impact of candidate genes described in the past in psychiatric disorders. Here we performed analyses using large publicly available datasets investigating a range of mutational mechanisms which impact variability of CNTNAP2 across several psychiatric disorders. In conclusion, our results converge to show a limited or likely neutral role of CNTNAP2 in the susceptibility of psychiatric disorders. However, the impact of this gene in language deficit per se is not directly examined in this study and warrants additional investigation.
Methods

Common variant association in CNTNAP2 using publicly available datasets

We sought to replicate previously reported CNTNAP2 SNP associations in a range of psychiatric phenotypes or traits using GWAS summary-statistic data of the Psychiatric Genomics Consortium (https://med.unc.edu/pgc/results-and-downloads).

Firstly, we report the corresponding P-values of specific previously associated markers for case-control cohorts with autism spectrum disorder (ASD), schizophrenia (SCZ), bipolar disorder (BD), attention-deficit hyperactivity-disorder (ADHD), major depressive disorder (MDD), anorexia nervosa (AN), and obsessive compulsive disorder (OCD). If a specific SNP marker was not reported in an individual GWAS dataset, we selected another marker in high linkage disequilibrium ($r^2\approx1$, using genotype data from the CEU, TSI, GBR and IBS European populations in 1000genomes project; http://www.internationalgenome.org).

Next, a gene-based association for common variants was calculated with MAGMA [86], using variants within a 5 kb window upstream and downstream of CNTNAP2. Selected datasets were of European descent, derived from GWAS summary statistics of the Psychiatric Genomics Consortium (https://med.unc.edu/pgc/results-and-downloads): SCZ (33,640 cases and 43,456 controls), BD (20,352 cases and 31,358 controls), ASD (6,197 and 7,377 controls), ADHD (19,099 cases and 34,194 controls), MDD (9,240 cases and 9,519 controls), OCD (2,688 cases and 7,037 controls), and AN (3,495 cases and 10,982 controls) [87–93]. Analyses were performed combining two different models for higher statistical power and sensitivity when the genetic architecture is unknown: the combined P-value model, which is more sensitive when only a small proportion of key SNPs in a gene show association; and the mean SNP association, which is more sensitive when allelic heterogeneity is greater and a larger number of SNPs show nominal association.

Finally, we selected SNPs predicted to be functional within a 5kb window upstream/downstream of CNTNAP2 (e.g. located in transcription factor binding sites, miRNA binding sites etc; https://snpinfo.niehs.nih.gov), and assessed a potential cross-disorder effect using GWAS summary statistics data of the PGC by performing a meta-analysis in PLINK [94]. The Cochran’s Q-statistic and $I^2$ statistic were calculated to examine heterogeneity amongst studies. The null hypothesis was that all studies were measuring the same true effect, which would be rejected if heterogeneity exists across studies. For all functional SNPs, when heterogeneity between studies was I$^2>50\%$ ($P<0.05$), the pooled OR was estimated using a random-effects model.

Analysis of rare variants in CNTNAP2 in ASD and schizophrenia, and de novo variants across psychiatric cohorts

The impact of rare variants of CNTNAP2 was assessed using sequencing-level data from the following datasets: WES from the Sweden-Schizophrenia population-based Case-Control cohort (6,135 cases and 6,245 controls; dbGAP accession: phs000473.v2.p2); ARRA Autism Sequencing Collaboration (490 BCM cases, BCM 486 controls, and 1,288 unrelated ASD probands from consent code c1; dbGAP accession: phs000298.v3.p2); Medical Genome Reference Bank (2,845 healthy Australian adults; https://sgc.garvan.org.au/initiatives/mgrb); individuals from a Caucasian Spanish population (719 controls [95, 96]); in-house ASD patients (30 cases; [97]); and previous published dataset in ASD (2,704 cases and 2,747 controls [84]). The selection of potentially etiologic variants was performed based on their predicted pathogenicity (missense damaging in both SIFT and polyphen 2, canonical splice variants, stop mutation...
and indels) and minor allele frequency (MAF < 0.0001 in non-Finnish European populations using the Genome Aggregation Database; http://gnomad.broadinstitute.org/). A chi square statistic was used to compare separately the sample of schizophrenia patients (6,135 cases) and the combined ASD datasets (4,512 cases) with the combined control datasets (13,042 individuals).

Two databases for de novo variants were used to identify de novo variants in CNTNAP2 [98, 99], which comprise data for the following samples: autism spectrum disorder (6,171 families), schizophrenia (1,164 families), epilepsy (647 families), intellectual disability (1,101 families), developmental disorders (4,293 families) and controls (2,163).

Extended family with bipolar disorder and CNV in CNTNAP2

The extended family presented here (Fig 2B) provides a molecular follow-up from a previously reported whole exome sequencing (WES) study of multiplex BD families, augmented with CNV microarray data [78]. This multigenerational pedigree, was collected through the Mood Disorders Unit and Black Dog Institute at the Prince of Wales Hospital, Sydney, and the School of Psychiatry (University of New South Wales in Sydney) [100–104]. Consenting family members were assessed using the Family Interview for Genetic Studies (FIGS) [105], and the Diagnostic Interview for Genetic Studies (DIGS) [106]. The study was approved by the Human Research Ethics Committee of the University of New South Wales, and written informed consent was obtained from all participating individuals. Blood samples were collected for DNA extraction by standard laboratory methods. Three of the five relatives with bipolar disorder type I (BD-I) had DNA and WES-derived genotype data available, and six unaffected relatives with DNA and WES data were available for haplotype phasing and segregation analysis (Fig 2B).

Genome-wide CNV analysis was performed via CytoScan HD Array (Affymetrix, Santa Clara, CA, USA) in 2 distal affected relatives (individuals 8410 and 8401; Fig 2B), using the Affymetrix Chromosome Analysis Suite (ChAS) software (ThermoFisher, Waltham, MA, USA). Detailed information on CNV detection and filtering criteria have been previously described [78]. We identified a 131kb deletion in intron 1 of CNTNAP2 in individual 8401. WES-derived genotypes were used for haplotype assessment to infer CNV segregation amongst relatives, as previously described [78]. Next, we experimentally validated the CNTNAP2 CNV via quantitative PCR (qPCR) in all available family members. Validation was performed in quadruplicate via a SYBR Green-based quantitative PCR (qPCR) method using two independent amplicon probes, each compared with two different reference amplicon probes in the FOXP2 and RNF20 genes (S4 Table). Experimental details are available upon request.

Supporting information

S1 Fig. CNTNAP association plots using GWAS summary statistics of the PGC data sets. (DOCX)

S2 Fig. Cross-disorder association plot of CNTNAP2 common variants predicted to be functional (63 SNPs). (DOCX)

S1 Table. Gene-based analysis of predicted functional SNPs across seven psychiatric disorders. (DOCX)
S2 Table. Full list of de novo variants in CNTNAP2 gene.

(DOCX)

S3 Table. Full list of Ultra-Rare Variants (URVs) in available sequencing datasets.

(DOCX)

S4 Table. Primers used in the CNV validation for the CNTNAP2 intronic deletion.

(DOCX)

Acknowledgments

We thank Xose S. Puente from the University of Oviedo (Spain) for providing us the data from a Spanish control population. We are grateful to all participants and their families, as well as clinical collaborators who were originally involved in collecting and phenotyping these families, including Laila Tabassum, and Adam Wright (UNSW).

Author Contributions

Conceptualization: Claudio Toma, Janice M. Fullerton.

Data curation: Claudio Toma, Alex D. Shaw.

Formal analysis: Claudio Toma, Alex D. Shaw.

Funding acquisition: Philip B. Mitchell, Peter R. Schofield, Janice M. Fullerton.

Investigation: Claudio Toma, Kerrie D. Pierce, Alex D. Shaw, Anna Heath, Janice M. Fullerton.

Methodology: Claudio Toma, Janice M. Fullerton.

Project administration: Janice M. Fullerton.

Resources: Claudio Toma, Philip B. Mitchell, Peter R. Schofield, Janice M. Fullerton.

Software: Claudio Toma, Alex D. Shaw.

Supervision: Claudio Toma, Janice M. Fullerton.

Validation: Claudio Toma, Kerrie D. Pierce.

Visualization: Claudio Toma, Kerrie D. Pierce, Janice M. Fullerton.

Writing – original draft: Claudio Toma.

Writing – review & editing: Claudio Toma, Kerrie D. Pierce, Alex D. Shaw, Anna Heath, Philip B. Mitchell, Peter R. Schofield, Janice M. Fullerton.

References

1. Poliak S, Salomon D, Elhanany H, Sabanay H, Kiernan B, Pevny L, et al. Juxtaparanodal clustering of Shaker-like K+ channels in myelinated axons depends on Caspr2 and TAG-1. The Journal of cell biology. 2003; 162(6):1149–60. https://doi.org/10.1083/jcb.200305018 PMID: 12963709; PubMed Central PMCID: PMC2172860.

2. Penagarikano O, Abrahams BS, Herman EL, Winden KD, Gadisah A, Dong H, et al. Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits. Cell. 2011; 147(1):235–46. https://doi.org/10.1016/j.cell.2011.08.040 PMID: 21962519; PubMed Central PMCID: PMC3390029.

3. Abrahams BS, Tentler D, Perederiy JV, Oldham MC, Coppola G, Geschwind DH. Genome-wide analyses of human perisylvian cerebral cortical patterning. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104(45):17849–54. https://doi.org/10.1073/pnas.0706128104 PMID: 17978184; PubMed Central PMCID: PMC2077018.
4. Anderson GR, Galfin T, Xu W, Aoto J, Malenka RC, Sudhof TC. Candidate autism gene screen identifies critical role for cell-adhesion molecule CASPR2 in dendritic arborization and spine development. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109(44):18120–5. https://doi.org/10.1073/pnas.1216398109 PMID: 23074245; PubMed Central PMCID: PMC3497786.

5. Strauss KA, Puffenberger EG, Huentelman MJ, Gottlieb S, Dobrin SE, Parod JM, et al. Recessive symptomatic focal epilepsy and mutant contactin-associated protein-like 2. The New England journal of medicine. 2006; 354(13):1370–7. https://doi.org/10.1056/NEJMoa052773 PMID: 16571880.

6. Zweier C, de Jong EK, Zweier M, Orrico A, Ousager LB, Collins AL, 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. American journal of human genetics. 2009; 85(5):655–66. https://doi.org/10.1016/j.ajhg.2009.10.004 PMID: 19896112; PubMed Central PMCID: PMC2775834.

7. 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 medical genetics. 2016; 17:8. https://doi.org/10.1186/s12881-016-0272-8 PMID: 26843181; PubMed Central PMCID: PMC4739328.

8. Smogavec M, Cleall A, Hoyer J, Lederer D, Nassogne MC, Palmer EE, et al. Eight further individuals with intellectual disability and epilepsy carrying bi-allelic CNTNAP2 aberrations allow delineation of the mutational and phenotypic spectrum. Journal of medical genetics. 2016; 53(12):820–7. https://doi.org/10.1136/jmedgenet-2016-103880 PMID: 27439707.

9. Watson CM, Crippon LA, Tzika A, Mills A, Coates A, Pendlebury M, et al. Diagnostic whole genome sequencing and split-read mapping for nucleotide resolution breakpoint identification in CNTNAP2 deficiency syndrome. American journal of medical genetics Part A. 2014; 164A(10):2649–55. https://doi.org/10.1002/ajmg.a.36679 PMID: 25045150.

10. Kale T, Philip M. An Interstitial Deletion at 7q33-36.1 in a Patient with Intellectual Disability, Significant Language Delay, and Severe Microcephaly. Case reports in genetics. 2016; 2016:6046351. https://doi.org/10.1155/2016/6046351 PMID: 28053794; PubMed Central PMCID: PMC5178345.

11. 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(6855):519–23. https://doi.org/10.1038/35097076 PMID: 11586359.

12. Vernes SC, Newbury DF, Abrahams BS, Winchester L, Nicod J, Groszer M, et al. A functional genetic link between distinct developmental language disorders. The New England journal of medicine. 2008; 359(22):2337–45. https://doi.org/10.1056/NEJMoa0802828 PMID: 18987363; PubMed Central PMCID: PMC2756409.

13. Alarcon M, Abrahams BS, Stone JL, Duvall JA, Perederiy JV, Bomar JM, et al. Linkage, association, and gene-expression analyses identify CNTNAP2 as an autism-susceptibility gene. American journal of human genetics. 2008; 82(1):150–9. https://doi.org/10.1016/j.ajhg.2007.09.005 PMID: 18179893; PubMed Central PMCID: PMC2253955.

14. Arking DE, Cutler DJ, Brune CW, Teslovich TM, West K, Ikeda M, et al. A common genetic variant in the neurexin superfamily member CNTNAP2 increases familial risk of autism. American journal of human genetics. 2008; 82(1):160–4. https://doi.org/10.1016/j.ajhg.2007.09.015 PMID: 18179894; PubMed Central PMCID: PMC2253968.

15. Whitehouse AJ, Bishop DV, Ang QW, Pennell CE, Fisher SE. CNTNAP2 variants affect early language development in the general population. Genes, brain, and behavior. 2011; 10(4):451–6. https://doi.org/10.1111/j.1601-183X.2011.00684.x PMID: 21310003; PubMed Central PMCID: PMC3130139.

16. Whalley HC, O’Connell G, Sussmann JE, Peel A, Stanfield AC, Haylou-Thomas ME, et al. Genetic variation in CNTNAP2 alters brain function during linguistic processing in healthy individuals. American journal of medical genetics Part B, Neuropsychiatric genetics: the official publication of the International Society of Psychiatric Genetics. 2011; 156B(8):941–8. https://doi.org/10.1002/ajmg.b.31241 PMID: 21987501.

17. Clemm von Hohenberg C, Wigand MC, Kubicki M, Leicht G, Giegling I, Karch S, et al. CNTNAP2 polymorphisms and structural brain connectivity: a diffusion-tensor imaging study. Journal of psychiatric research. 2013; 47(10):1349–56. https://doi.org/10.1016/j.jpsychires.2013.07.002 PMID: 23871450; PubMed Central PMCID: PMC3780783.

18. Scott-Van Zeeland AA, Abrahams BS, Alvarez-Retuerto AI, Sonnenblick LI, Rudie JD, Gahremani D, et al. Altered functional connectivity in frontal lobe circuits is associated with variation in the autism risk gene CNTNAP2. Science translational medicine. 2010; 2(56):56ra80. https://doi.org/10.1126/scitranslmed.3001344 PMID: 21048216; PubMed Central PMCID: PMC3065863.

19. Dennis EL, Jahanshad N, Rudie JD, Brown JA, Johnson K, McMahon KL, et al. Altered structural brain connectivity in healthy carriers of the autism risk gene, CNTNAP2. Brain Connect. 2011; 1(6):447–59.
Anney R, Klei L, Pinto D, Almeida J, Bacchelli E, Baird G, et al. Individual common variants exert weak
27.
Sampath S, Bhat S, Gupta S, O’Connor A, West AB, Arking DE, et al. Defining the contribution of
26.
Chiocchetti AG, Kopp M, Waltes R, Haslinger D, Duketis E, Jarczok TA, et al. Variants of the CNTNAP2
25.
Poot M. A candidate gene association study further corroborates involvement of contactin genes in
28.
Polimanti R, Squitti R, Pantaleo M, Giglio S, Zito G. Duplication of FOXP2 binding sites within
31.
Mikhail FM, Lose EJ, Robin NH, Descartes MD, Rutledge KD, Rutledge SL, et al. Clinically relevant
32.
Gregor A, Albrecht B, Bader I, Bijlsma EK, Ekici AB, Engels H, et al. Expanding the clinical spectrum
29.
Bakkaloglu B, O’Roak BJ, Louvi A, Gupta AR, Abelson JF, Morgan TM, et al. Molecular cytogenetic
33.
Belloso JM, Bache I, Guitart M, Caballin MR, Halgren C, Kirchhoff M, et al. Disruption of the CNTNAP2
30.
Steer CD, Golding J, Bolton PF. Traits contributing to the autistic spectrum. PloS one. 2010; 5(9):
e12633. https://doi.org/10.1371/journal.pone.0012633 PMID: 20836814; PubMed Central PMCID: PMC2935882.
36.
Nascimento PP, Bossolani-Martins AL, Rosan DB, Mattos LC, Brandao-Mattos C, Fett-Conte AC. Single
nucleotide polymorphisms in the CNTNAP2 gene in Brazilian patients with autistic spectrum disorder.
Genetics and molecular research: GMR. 2016; 15(1). https://doi.org/10.4238/gmr.15017422 PMID: 26909962.
24.
Chiocchetti AG, Kopp M, Waltes R, Haslinger D, Duketis E, Jarczok TA, et al. Variants of the CNTNAP2
5’ promoter as risk factors for autism spectrum disorders: a genetic and functional approach. Molecular
psychiatry. 2015; 20(7):839–49. https://doi.org/10.1038/mp.2014.103 PMID: 25224256.
26.
Sampath S, Bhat S, Gupta S, O’Connor A, West AB, Arking DE, et al. Defining the contribution of
CNTNAP2 to autism susceptibility. PloS one. 2013; 8(10):e77906. https://doi.org/10.1371/journal.
pone.0077906 PMID: 24147096; PubMed Central PMCID: PMC3798378.
21.
Koeda M, Watanabe A, Tsuda K, Matsumoto M, Ikeda Y, Kim W, et al. Interaction effect between
handedness and CNTNAP2 polymorphism (rs7794745 genotype) on voice-specific frontotemporal
activity in healthy individuals: an fMRI study. Frontiers in behavioral neuroscience. 2015; 9:87. https://
doi.org/10.3389/fnbeh.2015.00087 PMID: 25941478; PubMed Central PMCID: PMC4403548.
23.
Steer CD, Golding J, Bolton PF. Traits contributing to the autistic spectrum. PloS one. 2010; 5(9):
e12633. https://doi.org/10.1371/journal.pone.0012633 PMID: 20836814; PubMed Central PMCID: PMC2935882.
35.
Girirajan S, Dennis MY, Baker C, Malig M, Coe BP, Campbell CD, et al. Refinement and discovery of
new hotspots of copy-number variation associated with autism spectrum disorders. American journal of
human genetics. 2010; 82(1):165–73. https://doi.org/10.1016/j.ajhg.2007.09.017 PMID: 17392702.
34.
Prasad A, Merico D, Thiruvahindrapuram B, Wei J, Lionel AC, Sato D, et al. A discovery resource of
rare copy number variations in individuals with autism spectrum disorder. G3. 2012; 2(12):1665–85.
https://doi.org/10.1534/g3.112.004689 PMID: 23275889; PubMed Central PMCID: PMC3516488.
37. Nord AS, Roeb W, Dickel DE, Walsh T, Kusenda M, O’Connor KL, et al. Reduced transcript expression of genes affected by inherited and de novo CNVs in autism. European journal of human genetics: EJHG. 2011; 19(6):727–31. https://doi.org/10.1038/ejhg.2011.24 PMID: 21448237; PubMed Central PMCID: PMC3110052.

38. Egger G, Roetzer KM, Noor A, Lionel AC, Mahmood H, Schwarzbrun T, et al. Identification of risk genes for autism spectrum disorder through copy number variation analysis in Austrian families. Neurogenetics. 2014; 15(2):117–27. https://doi.org/10.1007/s10048-014-0394-0 PMID: 24843514.

39. Vogt D, Cho KKA, Shelton SM, Paul A, Huang ZJ, Sohal VS, et al. Mouse Cntnap2 and Human CNTNAP2 ASD Alleles Cell Autonomously Regulate PV+ Cortical Interneurons. Cerebral cortex. 2017:1–12. https://doi.org/10.1093/cercor/bhx248 PMID: 29028946.

40. Scott R, Sanchez-Aguilera A, van Elst K, Lim L, Dehorter N, Bae SE, et al. Loss of Cntnap2 Causes Axonal Excitability Deficits, Developmental Delay in Cytomyelination, and Abnormal Stereotyped Motor Behavior. Cerebral cortex. 2017. https://doi.org/10.1093/cercor/bhx341 PMID: 29300891.

41. Wang KS, Liu XF, Aragam N. A genome-wide meta-analysis identifies novel loci associated with schizophrenia and bipolar disorder. Schizophrenia research. 2010; 124(1–3):192–9. https://doi.org/10.1016/j.schres.2010.09.002 PMID: 20889312.

42. Zhong X, Zhang H. Linkage analysis and association analysis in the presence of linkage using age at onset of COGA alcoholism data. BMC genetics. 2005; 6 Suppl 1:S31. https://doi.org/10.1186/1471-2156-6-S1-S31 PMID: 16451641; PubMed Central PMCID: PMC1866754.

43. Terracciano A, Sanna S, Uda M, Deiana B, Usala G, Busonero F, et al. Genome-wide association scan for five major dimensions of personality. Molecular psychiatry. 2010; 15(6):647–56. https://doi.org/10.1038/mp.2008.113 PMID: 18957941; PubMed Central PMCID: PMC2874623.

44. Wray NR, Pergadia ML, Blackwood DH, Penninx BW, Gordon SD, Nyholt DR, et al. Genome-wide association study of major depressive disorder: new results, meta-analysis, and lessons learned. Molecular psychiatry. 2012; 17(1):36–48. https://doi.org/10.1038/mp.2010.109 PMID: 21042317; PubMed Central PMCID: PMC3252611.

45. Chen X, Long F, Cai B, Chen X, Chen G. A novel relationship for schizophrenia, bipolar and major depressive disorder Part 7: A hint from chromosome 7 high density association screen. Behavioural brain research. 2015; 293:441–51. https://doi.org/10.1016/j.bbr.2015.06.043 PMID: 26192912.

46. Newbury DF, Paracchini S, Scerri TS, Winchester L, Addis L, Richardson AJ, et al. Investigation of dyslexia and SLI risk variants in reading- and language-impaired subjects. Behavior genetics. 2011; 41(1):90–104. https://doi.org/10.1007/s10519-010-9424-3 PMID: 21165619; PubMed Central PMCID: PMC3029677.

47. Peter B, Raskind WH, Matsushima M, Lisowski M, Vu T, Berninger VW, 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. Journal of neurodevelopmental disorders. 2011; 3(1):39–49. https://doi.org/10.1007/s11668-010-9065-0 PMID: 21484596; PubMed Central PMCID: PMC3163991.

48. Stein MB, Yang BZ, Chavira DA, Hitchcock CA, Sung SC, Shipon-Blum E, et al. A common genetic variant in the neurexin superfamily member CNTNAP2 is associated with increased risk for selective mutism and social anxiety-related traits. Biological psychiatry. 2011; 69(9):825–31. https://doi.org/10.1016/j.biopsych.2010.11.008 PMID: 21193173; PubMed Central PMCID: PMC3079072.

49. Ji W, Li T, Pan Y, Tao H, Ju K, Wen Z, et al. CNTNAP2 is significantly associated with schizophrenia and major depression in the Han Chinese population. Psychiatry research. 2013; 207(3):225–8. https://doi.org/10.1016/j.psychres.2012.09.024 PMID: 23123147.

50. Zhao YJ, Wang YP, Yang WZ, Sun HW, Ma HW, Zhao YR. CNTNAP2 Is Significantly Associated With Speech Sound Disorder in the Chinese Han Population. Journal of child neurology. 2015; 30(13):1806–11. https://doi.org/10.1177/0883073815581609 PMID: 25895914.

51. Friedman JL, Vrijenhoek T, Marks S, Janssen IM, van der Vliet WA, Faas BH, et al. CNTNAP2 gene dosage variation is associated with schizophrenia and epilepsy. Molecular psychiatry. 2008; 13(3):261–6. https://doi.org/10.1038/mp.2007.74 PMID: 17648849.

52. Malhotra D, McCarthy S, Michaelson JJ, Vacie V, Burdick KE, Yoon S, et al. High frequencies of de novo CNVs in bipolar disorder and schizophrenia. Neuron. 2011; 72(6):951–63. https://doi.org/10.1016/j.neuron.2011.11.007 PMID: 22193173; PubMed Central PMCID: PMC3292677.

53. Zhang D, Cheng L, Qian Y, Allevy-Rodrigue N, Kelsoe JR, Greenwood T, et al. Singleton deletions throughout the genome increase risk of bipolar disorder. Molecular psychiatry. 2009; 14(4):376–80. https://doi.org/10.1038/mp.2008.144 PMID: 19114987; PubMed Central PMCID: PMC2735188.

54. Lee IS, Carvalho CM, Douvaras P, Ho SM, Hartley BJ, Zuccherato LW, et al. Characterization of molecular and cellular phenotypes associated with a heterozygous CNTNAP2 deletion using patient-derived hiPSC neural cells. NPJ schizophrenia. 2015; 1. https://doi.org/10.1038/npj schizophrenia.2015.19 PMID: 26985448; PubMed Central PMCID: PMC4789165.
55. Elia J, Gai X, Xie HM, Perin JC, Geiger E, Giessen JT, et al. Rare structural variants found in attention-deficit hyperactivity disorder are preferentially associated with neurodevelopmental genes. Molecular psychiatry. 2010; 15(6):637–46. https://doi.org/10.1038/mp.2009.57; PMID: 19546859; PubMed Central PMCID: PMC2877197.

56. Melford HC, Muhle H, Ostertag P, von Spiczak S, Buysse K, Barker C, et al. Genome-wide copy number variation in epilepsy: novel susceptibility loci in idiopathic generalized and focal epilepsies. PLoS genetics. 2010; 6(5):e1000962. https://doi.org/10.1371/journal.pgen.1000962; PubMed Central PMCID: PMC2873910.

57. Lesca G, Rudolf G, Labalme A, Hirsch E, Arzimanoglou A, Genton P, et al. Epileptic encephalopathies of the Landau-Kleffner and continuous spike and waves during slow-wave sleep types: genomic dissection makes the link with autism. Epilepsia. 2012; 53(9):1526–38. https://doi.org/10.1111/j.1528-1167.2012.03559.x; PMID: 22738016.

58. Curran S, Ahn JW, Grayton H, Collier DA, Ogilvie CM. NRXN1 deletions identified by array comparative genome hybridisation in a clinical case series—further understanding of the relevance of NRXN1 to neurodevelopmental disorders. Journal of molecular psychiatry. 2013; 1(1):4. https://doi.org/10.1186/2049-9256-1-4; PubMed Central PMCID: PMC4223877.

59. Pippucci T, Licchetta L, Baldassari S, Palombo F, Menghi V, D’Aurizio R, et al. Epilepsy with auditory features: A heterogenous clinico-molecular disease. Neurology Genetics. 2015; 1(1):e5. https://doi.org/10.1212/NXG.0000000000000005; PMID: 27066454; PubMed Central PMCID: PMC4921078.

60. Verkerk AJ, Mathews CA, Joosse M, Eussen BH, Heutink P, Oostra BA, et al. CNTNAP2 is disrupted in a family with Gilles de la Tourette syndrome and obsessive compulsive disorder. Genomics. 2003; 82(1):1–9. PMID: 12809671.

61. Hoyer H, Braathen GJ, Eek AK, Nordang GB, Skjelbred CF, Russell MB. Copy number variations in a population-based study of Charcot-Marie-Tooth disease. BioMed research international. 2015; 2015:960404. https://doi.org/10.1155/2015/960404; PMID: 25648254; PubMed Central PMCID: PMC4306395.

62. Al-Murrah A, Ashton F, Affimos S, George AM, Love DR. Amino-Terminal Microdeletion within the CNTNAP2 Gene Associated with Variable Expressivity of Speech Delay. Case reports in genetics. 2012; 2012:172408. https://doi.org/10.1155/2012/172408; PMID: 23074684; PubMed Central PMCID: PMC3447720.

63. Centanni TM, Sanmann JN, Green JR, Iuzzini-Seigel J, Bartlett C, Sanger WG, et al. The role of candidate-gene CNTNAP2 in childhood apraxia of speech and specific language impairment. American journal of medical genetics Part B, Neuropsychiatric genetics: the official publication of the International Society of Psychiatric Genetics. 2015; 168(7):536–43. https://doi.org/10.1002/ajmg.b.32325; PMID: 26097074.

64. Laffin JJ, Raca G, Jackson CA, Strand EA, Jakielski KJ, Shriberg LD. Novel candidate genes and regions for childhood apraxia of speech identified by array comparative genomic hybridization. Genetics in medicine: official journal of the American College of Medical Genetics. 2012; 14(11):928–36. https://doi.org/10.1038/gim.2012.72; PMID: 22766611; PubMed Central PMCID: PMC3563158.

65. Veerappa AM, Saldanha M, Padakannaya P, Ramachandra NB. Family-based genome-wide copy number scan identifies five new genes of dyslexia involved in dendritic spinal plasticity. Journal of human genetics. 2013; 58(8):539–47. https://doi.org/10.1038/jhg.2013.47; PMID: 23677055.

66. Chang YT, Chen PC, Tsai IJ, Sung FC, Chin ZN, Kuo HT, et al. Bidirectional relation between schizophrenia and epilepsy: a population-based retrospective cohort study. Epilepsia. 2011; 52(11):2036–42. https://doi.org/10.1111/j.1528-1167.2011.03268.x; PMID: 21929680.

67. Chang HJ, Liao CC, Hu CJ, Shen WW, Chen TL. Psychiatric disorders after epilepsy diagnosis: a population-based retrospective cohort study. PloS one. 2013; 8(4):e59999. https://doi.org/10.1371/journal.pone.0059999; PMID: 23577079; PubMed Central PMCID: PMC3618118.

68. Sucksdorf D, Brown AS, Chudal R, Jokiranta-Olkoniemi E, Leivonen S, Suominen A, et al. Parental and comorbid epilepsy in persons with bipolar disorder. Journal of affective disorders. 2015; 188:107–11. https://doi.org/10.1016/j.jad.2015.08.051; PMID: 26356289; PubMed Central PMCID: PMC4631649.

69. Wiglus MS, Landowski J, Cubala WJ, Agius M. Overlapping phenomena of bipolar disorder and epilepsy—a common pharmacological pathway. Psychiatry Danubina. 2015; 27 Suppl 1: S177–81. PMID: 26417756.

70. Tuchman R, Rapin I. Epilepsy in autism. The Lancet Neurology. 2002; 1(6):352–8. PMID: 12849396.

71. Besag FM. Epilepsy in patients with autism: links, risks and treatment challenges. Neuropsychiatric disease and treatment. 2018; 14:1–10. https://doi.org/10.2147/NDT.S120509; PMID: 29296085; PubMed Central PMCID: PMC5739118.

72. Wells R, Swaminathan V, Sundram S, Weinberg D, Bruggemann J, Jacomb I, et al. The impact of pre-morbid and current intellect in schizophrenia: cognitive, symptomatic, and functional outcomes. NPJ...
Role of CNTNAP2 gene in psychiatry

73. Pawelczyk A, Kotlicka-Antczak M, Lojek E, Ruszpel A, Pawelczyk T. Schizophrenia patients have higher-order language and extralinguistic impairments. Schizophrenia research. 2018; 192:274–80. https://doi.org/10.1016/j.schres.2017.04.030 PMID: 28438437.

74. Raucher-Chene D, Achim AM, Kaladjian A, Besche-Richard C. Verbal fluency in bipolar disorders: A systematic review and meta-analysis. Journal of affective disorders. 2017; 207:359–66. https://doi.org/10.1016/j.jad.2016.09.039 PMID: 27744224.

75. Rapin I, Dunn M. Update on the language disorders of individuals on the autistic spectrum. Brain & development. 2003; 25(3):166–72. PMID: 12689694.

76. Tager-Flusberg H, Calkins S, Nolin T, Baumberger T, Anderson M, Chadwick-Dias A. A longitudinal study of language acquisition in autistic and Down syndrome children. Journal of autism and developmental disorders. 1990; 20(1):1–21. PMID: 2139024.

77. Fromer M, Pocklington AJ, Kavanagh DH, Williams HJ, Dwyer S, Gormley P, et al. De novo mutations in schizophrenia implicate synaptic networks. Nature. 2014; 506(7487):179–84. https://doi.org/10.1038/nature12929 PMID: 24463507; PubMed Central PMID: PMC4237002.

78. Toma C, Shaw AD, Alcock RJN, Heath A, Pierce KD, Mitchell PB, et al. An examination of multiple classes of rare variants in extended families with bipolar disorder. Translational psychiatry. 2018; 8(1):65. https://doi.org/10.1038/s41398-018-0113-y PMID: 29531218.

79. Huang N, Lee I, Marcotte EM, Hurles ME. Characterising and predicting haploinsufficiency in the human genome. PLoS genetics. 2010; 6(10):e1001154. https://doi.org/10.1371/journal.pgen.1001154 PMID: 20976243; PubMed Central PMID: PMC2954820.

80. Ross LA, Del Bene VA, Molholm S, Jae Woo Y, Andrade GN, Abrahams BS, et al. Common variation in the autism risk gene CNTNAP2, brain structural connectivity and multisensory speech integration. Brain and language. 2017; 174:50–60. https://doi.org/10.1016/j.bandl.2017.07.005 PMID: 28738218.

81. Toma C, Hervas A, Torrico B, Balmana N, Salgado M, Maristany M, et al. Analysis of two language-related genes in autism: a case-control association study of FOXP2 and CNTNAP2. Psychiatric genetics. 2013; 23(2):82–5. https://doi.org/10.1097/YPG.0b013e32835d6f6c PMID: 23277129.

82. Werling AM, Bobrowski E, Taurines R, Gundelfinger R, Romanos M, Grunblatt E, et al. CNTNAP2 gene in high functioning autism: no association according to family and meta-analysis approaches. J Neural Transm (Vienna). 2016; 123(3):353–63. https://doi.org/10.1007/s00702-015-1458-5 PMID: 26559825.

83. Torrico B, Chiocchetti AG, Bacchelli E, Trabetti E, Hervas A, Franke B, et al. Lack of replication of previous autism spectrum disorder GWAS hits in European populations. Autism Res. 2017; 10(2):202–11. https://doi.org/10.1002/aur.1662 PMID: 27417655.

84. Murdoch JD, Gupta AR, Sanders SJ, Walker MF, Keaney J, Fernandez TV, et al. No evidence for significant overlap with schizophrenia implicate synaptic networks. Nature. 2014; 506(7487):179–84. https://doi.org/10.1038/nature12929 PMID: 24463507; PubMed Central PMID: PMC4237002.

85. Canali G, Garcia M, Hivert B, Pinatel D, Goullancourt A, Oguievskaiia K, et al. Genetic variants in autism-related CNTNAP2 impair axonal growth of cortical neurons. Human molecular genetics. 2018; 27(11):1941–54. https://doi.org/10.1093/hmg/ddy102 PMID: 29788201.

86. de Leeuw CA, Mooij JM, Heskes T, Posthuma D. MAGMA: generalized gene-set analysis of GWAS data. PLoS computational biology. 2015; 11(4):e1004219. https://doi.org/10.1371/journal.pcbi.1004219 PMID: 25885710; PubMed Central PMID: PMC4401657.

87. Demontis D, Walters RK, Martin J, Mattheisen M, Als TD, Agerbo E, et al. Discovery Of The First Genome-Wide Significant Risk Loci For ADHD. bioRxiv. 2017. https://doi.org/10.1101/145581.

88. Autism Spectrum Disorders Working Group of The Psychiatric Genomics C. Meta-analysis of GWAS of over 16,000 individuals with autism spectrum disorder highlights a novel locus at 10q24.32 and a significant overlap with schizophrenia. Molecular autism. 2017; 8:21. https://doi.org/10.1016/j.s41398-017-0137-9 PMID: 28540026; PubMed Central PMID: PMC5441062.

89. Major Depressive Disorder Working Group of the Psychiatric GC, Ripke S, Wray NR, Lewis CM, Hamilton SP, Weissman MM, et al. A mega-analysis of genome-wide association studies for major depressive disorder. Molecular psychiatry. 2013; 18(4):487–511. https://doi.org/10.1038/mp.2012.21 PMID: 22478276; PubMed Central PMID: PMC3837431.

90. Schizophrenia Working Group of the Psychiatric Genomics C. Biological insights from 108 schizophrenia-associated genetic loci. Nature. 2014; 511(7510):421–7. https://doi.org/10.1038/nature13595 PMID: 25056061; PubMed Central PMID: PMC4112379.
91. Stahl E, Forstner A, McQuillin A, Ripke S, Ophoff R, Scott L, et al. Genomewide association study identifies 30 loci associated with bipolar disorder. bioRxiv. 2017. https://doi.org/10.1101/173062

92. International Obsessive Compulsive Disorder Foundation Genetics C, Studies OCDCGA. Revealing the complex genetic architecture of obsessive-compulsive disorder using meta-analysis. Molecular psychiatry. 2018; 23(5):1181–8. https://doi.org/10.1007/s00134-017-5197-0

93. Duncan L, Yilmaz Z, Gaspar H, Walters R, Goldstein J, Anttila V, et al. Significant Locus and Metabolic Genetic Correlations Revealed in Genome-Wide Association Study of Anorexia Nervosa. The American journal of psychiatry. 2017; 174(9):850–8. https://doi.org/10.1176/appi.ajp.2017.16121402 PMID: 28494655; PubMed Central PMCID: PMC5581217.

94. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. American journal of human genetics. 2007; 81(3):559–75. https://doi.org/10.1086/519795 PMID: 17701901; PubMed Central PMCID: PMC1950838.

95. Dopazo J, Amadoz A, Bleda M, Garcia-Alonso L, Aleman A, Garcia-Garcia F, et al. Significant Locus and Metabolic Genetic Correlations Revealed in Genome-Wide Association Study of Anorexia Nervosa. The American journal of psychiatry. 2017; 174(9):850–8. https://doi.org/10.1176/appi.ajp.2017.16121402 PMID: 28494655; PubMed Central PMCID: PMC5581217.

96. Puente XS, Bea S, Valdes-Mas R, Villamor N, Gutierrez-Abri J, Martin-Subero Ji, et al. Non-coding recurrent mutations in chronic lymphocytic leukaemia. Nature. 2015; 526(7574):519–24. https://doi.org/10.1038/nature14666 PMID: 26200345.

97. Li J, Cai T, Jiang Y, Chen H, He X, Chen C, et al. Genes with de novo mutations are shared by four neuropsychiatric disorders discovered from NPdenovo database. Molecular psychiatry. 2016; 21(2):290–7. https://doi.org/10.1038/mp.2015.40 PMID: 25849321; PubMed Central PMCID: PMC4837654.

98. Turner TN, Yi Q, Krumm N, Huddleston J, Hoekzema K, HA FS, et al. denovo-db: a compendium of human de novo variants. Nucleic acids research. 2017; 45(D1):D804–D11. https://doi.org/10.1093/nar/gkw865 PMID: 27907889; PubMed Central PMCID: PMC5210614.

99. Fullerton JM, Donald JA, Mitchell PB, Schofield PR. Two-Dimensional Genome Scan Identifies Multiple Genetic Interactions in Bipolar Affective Disorder. Biol Psychiatry. 2010; 67(5):478–86. https://doi.org/10.1016/j.biopsych.2009.10.022 PMID: 20022591.

100. Fullerton JM, Liu Z, Badenhop RF, Schofield PR. Two-Dimensional Genome Scan Identifies Multiple Genetic Interactions in Bipolar Affective Disorder. Biol Psychiatry. 2010; 67(5):478–86. https://doi.org/10.1016/j.biopsych.2009.10.022 PMID: 20022591.

101. Badenhop RF, Moses MJ, Scimone A, Adams LJ, Kwok JB, Jones AM, et al. Genetic refinement and physical mapping of a 2.3 Mb probable disease region associated with a bipolar affective disorder susceptibility locus on chromosome 4q35. Am J Med Genet B Neuropsychiatr Genet. 2003; 117(1):23–32. https://doi.org/10.1002/ajmg.b.10023 PMID: 12555231.

102. National Institute of Mental Health. NIMH Genetics Initiative: Family Interview for Genetic Studies (FIGS). Rockville, MD1992.

103. Nurnberger Jr, Blehar MC, Kaufmann CA, York-Cooler C, Simpson SG, Harkavy-Friedman J, et al. Diagnostic interview for genetic studies. Rationale, unique features, and training. NIMH Genetics Initiative. Archives of general psychiatry. 1994; 51(11):849–59; discussion 63–4. PMID: 7944874.