Candidate Genes for Inherited Autism Susceptibility in the Lebanese Population

Silva Kourtian1,2,* Jihane Soueid1,4,*, Nadine J. Makhoul1, Dikran Richard Guisso1, Maria Chahrour3 & Rose-Mary N. Boustany1,4

Autism spectrum disorder (ASD) is characterized by ritualistic-repetitive behaviors and impaired verbal/non-verbal communication. Many ASD susceptibility genes implicated in neuronal pathways/brain development have been identified. The Lebanese population is ideal for uncovering recessive genes because of shared ancestry and a high rate of consanguineous marriages. Aims here are to analyze for published ASD genes and uncover novel inherited ASD susceptibility genes specific to the Lebanese. We recruited 36 ASD families (ASD: 37, unaffected parents: 36, unaffected siblings: 33) and 100 unaffected Lebanese controls. Cytogenetics 2.7 M Microarrays/CytoScan™ HD arrays allowed mapping of homozygous regions of the genome. The CNTNAP2 gene was screened by Sanger sequencing. Homozygosity mapping uncovered DPP4, TRHR, and MLF1 as novel candidate susceptibility genes for ASD in the Lebanese. Sequencing of hot spot exons in CNTNAP2 led to discovery of a 5 bp insertion in 23/37 ASD patients. This mutation was present in unaffected family members and unaffected Lebanese controls. Although a slight increase in number was observed in ASD patients and family members compared to controls, there were no significant differences in allele frequencies between affecteds and controls (C/TTCTG: $\chi^2$ value = 0.014; p = 0.904). The CNTNAP2 polymorphism identified in this population, hence, is not linked to the ASD phenotype.

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by repetitive/restrictive behaviors, problems in social interactions, and difficulties in communication. Even though the age of onset may be as early as 6 months, ASD is typically diagnosed after 18 months of age. Prevalence estimates of ASD in the general population have increased over the last 20 years. In 2010, 1 in 68 children in the USA was documented as affected, with 1 in 42 boys and 1 in 189 girls diagnosed with ASD. Small scale studies in Middle Eastern countries, including the gulf region and Saudi Arabia, found that in the latter 12.5% of children under the age of 18 years have ASD. In Lebanon, the load of pediatric patients with ASD is increasing, and recently an incidence of 1 in 66 was reported in the greater Beirut and Mount Lebanon areas, with affected male to affected female ratios reported as 1:1.01. In the past decade, the high concordance in monozygotic twins (>95%) compared to dizygotic twin pairs (22–65%) pointed to underlying genetic etiologies for ASD. Several factors had hindered identification of disease genes such as clinical variability, genetic heterogeneity, and contribution of environmental as well as epigenetic factors. Recurrent copy number variants (CNVs), however, have been identified, such as the maternally derived duplication of chromosome 15q11.2–13 identified in 0.5–3% of ASD cases. Moreover, ASD phenotypes often occur in monogenic neurodevelopmental syndromes such as Tuberous Sclerosis, Fragile X, and Rett syndrome. These have autism as one of their main clinical features but only account for 2–5% of autism cases. Under the new DSM-5 diagnostic criteria they have now been dropped from the umbrella of ASD. More recent whole exome sequencing studies, identified de novo mutations in up to 15% of ASD cases. Both CNVs and point mutations in genes such as CNTNAP2, SHANK3, NLGN3, NLGN4, and NRXN1 have been reported in cases of ASD.

1American University of Beirut Medical Center Special Kids Clinic, Neurogenetics Program and Division of Pediatric Neurology, Lebanon. 2Department of Biological and Environmental Sciences, Faculty of Science, Beirut Arab University, Lebanon. 3Eugene McDermott Center for Human Growth and Development, Departments of Neuroscience and Psychiatry, University of Texas Southwestern Medical Center, Dallas, Texas, USA. 4Biochemistry and Molecular Genetics, American university of Beirut, Lebanon. *These authors contributed equally to this work. Correspondence and requests for materials should be addressed to R.-M.N.B. (email: rb50@aub.edu.lb)
The genetic makeup of the Lebanese population is unique due to significant shared ancestry, and DNA recombination events occurring over millennia, all these accompanied by high rates of consanguinity. The distinctive genetic fingerprint of the Lebanese population is well suited for comparative genomic hybridization (CGH) microarray analysis, homozygosity mapping, and CNV analysis for discovery of novel as well as previously described recessive disease-causing genes in ASD. Array CGH has improved the identification of putative ASD genes and increased molecular diagnosis in children with ASD to 25%. Demarcation of runs of homozygosity in family members allows immediate exclusion of thousands of genes as potentially causative. Homozygosity mapping in pedigrees with shared ancestry provides a fruitful methodology to identify whether genes considered under an autosomal recessive disease model can lead to ASD17,18. Genome-wide analysis locates genetic variations and identifies disease loci by genotyping parents, affected, and unaffected progeny. Disease loci appear in regions where only affected individuals are homozygous for the suspected culprit allele. In the present study, microarray analysis was used to identify runs of homozygosity (ROH) unique to affected ASD patients. The occurrence of identical ROH regions in at least 5 out of 37 affected individuals shed light on novel ASD susceptibility candidate genes in these regions. One of the genes that were common to 9 ROH regions in our cohort is CNTNAP2. It encodes a neuronal cell adhesion molecule that interacts with Contactin 2 (Cntn2) at the juxtaparanodal region at the nodes of Ranvier. These are regularly spaced gaps between the myelin-producing Schwann cells in the peripheral nervous system (PNS)19,20. While previous investigations have focused on the role of CNTNAP2 in PNS development, a recent report demonstrated that a homozygous CNTNAP2 mutation in the Old Order Amish population results in intractable seizures, histologically confirmed cortical neuronal migration abnormalities, and ASD21. It is hypothesized that ASD may result from improper excitatory/inhibitory balance brought by the inability to form adequate synaptic connections22. We screened CNTNAP2 for mutations in our cohort of ASD patients, their family members, and unaffected controls, and identified a novel polymorphism in the Lebanese population.

Results
All affected patients satisfied DSM4/5 criteria and were evaluated by a pediatric neurologist, pediatric psychiatrist and psychologists. Family history and clinical assessment of the phenotypic features are summarized in Table 1. Microarray analysis and mapping of ROH were performed in each family. Assuming a model of autosomal recessive inheritance and high penetrance, only ROHs unique to the affected patient(s) were considered (Table 1).

Of these ROH, only those found in 5 or more out of the 37 affected children were analyzed, particularly if they were overlapping ROH in different patients/families (Table 2). We found one stretch of ROH common to 5 children with ASD, located on chromosome 8q23.1, two ROH common to 6 children with ASD, one located on chromosome 2p22.1 and the other located on chromosome 3q25.32, and one ROH common to 7 children with ASD located on chromosome 18q11.2. ASD-associated copy number variations spanning these ROH regions in part, or whole, of all 4 ROH regions listed below have been reported in individuals with autism in the Simons Foundation Autism Research Initiative (SFARI) gene database.

Pathway analysis of the genes within these 4 ROH regions did not reveal relationships amongst those genes, but linked one of them (TRHR) to neurological diseases including seizures, anxiety, and neuronal hyperexcitability (Fig. 1). The genes were involved in different cell processes such as apoptosis, cell differentiation, cell proliferation, DNA damage, RNA splicing, inflammatory response, endocytosis, cell growth, vascularization, and lipid storage. Only TRHR and MLF1 implicated cell processes related to the brain. In addition to these 2 genes, the analysis of ROH regions common to at least 4 ASD patients uncovered more genes (data not shown), one of them, DPP4, was of particular interest. We also identified additional nine ROH found in 7 ASD patients that were not completely overlapping, but they all occurred within one gene, CNTNAP2 (Fig. 2). Network analysis of TRHR, MLF1, DPP4, and CNTNAP2 showed relationships of these genes to neuronal processes including endocytosis, neurite outgrowth, synaptic transmission, neuronal death as well as neuron development and neurite outgrowth, regulation of action potential and synaptic transmission, in addition to brain and neuron development (Fig. 3). Both the newly identified DPP4, previously linked to schizophrenia and brain dysfunction, and TRHR, previously linked to neuronal hyperexcitability, seizures, and anxiety may be linked to ASD in our cohort.

CNTNAP2 is the only gene to be previously reported in ASD. We performed mutational screening for CNTNAP2 in our cohort, and only exons previously reported as harboring mutations were screened (exons 8, 9, 10, 11, 12, 13, 14, 17, 20, 21, 22, 23, and 24). A 5 bp insertion (Fig. 4, red arrow) was detected in the intron upstream of exon 23 of CNTNAP2. This mutation was found in 23 out of 37 unrelated ASD patients we screened. Eight ASD patients were homozygous for this insertion and fifteen ASD patients were heterozygous (Table 3).

Genotyping for CNTNAP2 in our cohort of 106 total individuals consisting of ASD patients and their family members revealed 32 (30.2%) individuals with the NN genotype (homozygous for the reference allele), 44 (41.5%) individuals with the NV genotype, and 30 (28.3%) individuals with theVV genotype (homozygous for the alternate allele) (Table 3). Moreover, the screening of 100 unaffected control individuals with no family history of ASD uncovered 34 (34%) individuals with the NN genotype, 50 (50%) individuals with the NV genotype, and 16 (16%) individuals with the VV genotype (Table 3). There was no significant difference between ASD families and the control group for this polymorphism (value = 4.5335; p = 0.103). The alternate (V) allele frequencies in cases and controls were 0.49 and 0.41, respectively, however, this difference was not statistically significant ( value = 0.014; p = 0.904). Furthermore, this particular variant (chr7: 148,106,477 C > TTCTG) was absent from 60,706 unrelated individuals in the Exome Aggregation Consortium (ExAC) server. Since the majority of ExAC data pertains to individuals of European ancestry, it is not surprising that our variant identified in a Lebanese cohort was absent from ExAC.
**Patient ID** | **Age** | **Gender** | **Total Number of ROH in the genome** | **Locus of overlapping ROH regions** | **Satisfied DSM4/5 criteria** | **Family history** | **Additional notable clinical features**
--- | --- | --- | --- | --- | --- | --- | ---
F1-001 | 2y | Male | 65 | 2p22.1 | + | Negative for psychiatric/mental disorder | Tip-toe walking, non-verbal
F2-005 | 14y | Male | 29 | + | Mental retardation in mother’s uncle | Non-verbal, ECAR-T score = 61
F3-010 | 23y | Male | 68 | 2p22.1 18q12.1 | + | Negative for psychiatric/mental disorder | N.A.
F4-015 | 17y | Male | 44 | 8q23.1 3q25.32 18q12.1 | + | Negative for psychiatric/mental disorder | ECAR-T score = 31
F5-020 | 5y | Male | 50 | + | Depression in maternal grand-father | N.A.
F6-024 | 10y | Male | 41 | 2p22.1 8q23.1 3q25.32 | + | Negative for psychiatric/mental disorder | N.A.
F7-029 | 21y | Male | 35 | 8q23.1 | + | Asperger’s case in maternal family | N.A.
F8-034 | 14y | Male | 77 | + | Negative for psychiatric/mental disorder | N.A.
F9-038 | 10y | Male | 113 | 8q23.1 | + | Negative for psychiatric/mental disorder | N.A.
F11-048 | 7y | Male | 36 | + | Negative for psychiatric/mental disorder | Verbal, talked at 2y
F15-070 | 6y | Male | 11 | + | Negative for psychiatric/mental disorder | Agoraphobia
F17-080 | 2y | Male | 59 | + | Father has autistic features | Hyperactive, speech delay
F18-083 | 13y | Male | 45 | 2p22.1 | + | Negative for psychiatric disorder. Mental retardation in a family member | Speech delay
F19-088 | 4y | Male | 11 | 2p22.1 | + | Negative for psychiatric disorder. Mental retardation in a family member | High IQ, speech regression at 2.5y, aggressive, mild ASD
F19-089 | 3y | Male | 11 | 2p22.1 | + | Negative for psychiatric/mental disorder | Severe autistic features, echolalia, speech regression at 2y, mental retardation
F20-093 | 3y | Male | 49 | 3q25.32 18q12.1 | + | Negative for psychiatric/mental disorder | Speech regression at 2y, stereotypies, little improvement with therapies
F22-101 | 5y | Male | 47 | 18q12.1 | + | Mental retardation in paternal cousin | Congenital cataract, Hyperactivity
F24-108 | 8y | Male | 5 | + | Negative for psychiatric/mental disorder | Speech regression at 2y
F27-122 | 8y | Male | 35 | 3q25.32 | + | Negative for psychiatric/mental disorder | N.A.
F28-127 | 9y | Male | 29 | 3q25.32 18q12.1 | + | Negative for psychiatric/mental disorder | N.A.
F29-133 | 8y | Male | 41 | 2p22.1 | + | Negative for psychiatric/mental disorder | N.A.
F31-145 | 3y | Male | 47 | + | Negative for psychiatric/mental disorder | Hyperactive, stereotypies, speech delay
F32-148 | 8y | Male | 38 | + | Negative for psychiatric/mental disorder | Speech delay at 3.5y, significant improvement with therapies
F33-152 | 2y | Male | 31 | + | Negative for psychiatric/mental disorder | Non-verbal, tip toe walking
F34-158 | 4y | Male | 10 | + | Negative for psychiatric/mental disorder | No stereotypical movements, aggressive, small improvement with therapies
F35-164 | 16y | Female | 32 | 18q12.1 | + | Negative for psychiatric/mental disorder | Non-verbal, hyperactive
F36-169 | 4y | Male | 61 | 3q25.32 18q12.1 | + | Family history of autism | Non-verbal.
F37-172 | 8y | Male | 44 | + | Negative for psychiatric/mental disorder | Walked at 2.5y, non-verbal, stereotypies, Krabbe disease
F38-176 | 10y | Male | 37 | + | Negative for psychiatric/mental disorder | Delayed speech, walked at 1y 4 m, aggressive, improved with therapies
F39-180 | 8y | Male | 18 | + | Negative for psychiatric/mental disorder | Walked at 1.6y, few words, epilepsy
F40-186 | 5y | Male | 57 | 8q23.1 | + | Mother had intra-partum depression | Speech regression, rigid behavior, stereotypies
F41-190 | 15y | Male | 37 | + | Father has depression. Complications during labor and delivery | Talked at 1y5m, stereotypies, rigid behavior, echolalia
F42-194 | 7y | Male | 25 | + | Mother has depression | Stereotypies, non-verbal, delayed walking
F43-197 | N.A | Male | 26 | + | Negative for psychiatric/mental disorder | Feeding difficulties in early childhood, drooling, flapping tip toe walking, Non-verbal, improvement with therapies
F44-201 | 3y | Male | 20 | + | Negative for psychiatric/mental disorder | Speech delay, stereotypies
F45-205 | 12y | Male | 21 | + | Asperger’s case on the maternal side | Hyperactivity, non-verbal, improvement with therapies
F46-211 | N.A | Female | 51 | + | Negative for psychiatric/mental disorder | Regression at 2y, tip toe walking, stereotypies, non-verbal

**Table 1.** Characteristics of patients with autism included in the study. Total number of ROH in the genome are only ROHs unique to the affected patient(s) and not present in the parents (column 4). Locus of overlapping ROH regions found at least in 5 ASD patients and described in table 2 (see column 5). N.A: Data not available.
Discussion

We identified three novel candidate ASD genes, DPP4, TRHR, and MLF1, and network analysis provided arguments in favor of these genes. DPP4 is directly related to anxiety and schizophrenia. It plays a role in endocytosis and neuronal death as well as brain dysfunction. Although DPP4 had never been directly associated with autism, some findings link it to autistic features. Recent studies show that a decrease in the gene expression or activity of DPP4 would result in possible neurological consequences and exacerbation of autism symptoms. Moreover, a deletion encompassing DPP4 among other genes was found in a patient presenting with hypotonia, delayed motor development, severe language impairment, and behavior consistent with ASD. In another study, DPP4 was linked to attention problems and aggressiveness. Increased activity of DPP4 leads to a reduced amount of oxytocin and vasopressin, with low levels of these neuropeptides leading to increased aggressiveness and negative social behavior. In a rodent model, targeted inactivation of the Dpp4 gene resulted in healthy knockout mice. Dpp4−/− homozygous mutants showed hypoglycemia, hyperinsulinemia, and increased plasma glucagon-like peptide 1 in glucose tolerance tests. Behavior of these mice by analyzing “behavioral despair” is reported. This behavior consists of failure to escape from aversive stimuli such as anxiety, curiosity, and motor activity. This is similar behavior following chronic administration of antidepressants. Along with reduced depressive-like behavior, Dpp4−/− mice show higher novelty-induced hyperactivity. These reported findings impart a strong role for DPP4 expression in the regulation of mood.

The thyrotropin-releasing hormone receptors (TRHR) are found in the anterior pituitary and in neurons throughout the central nervous system. The neuroanatomical location and neurochemical actions of TRH and its receptors suggest that it could be utilized as a therapeutic agent in neurological and psychiatric disorders. Several studies of TRH effect in patients with depression reported an improvement in anxiety as well as in depressive mood. TRH may also protect against rather than induce seizures. It also demonstrates neuroprotective effects, and may be involved in chronic neuronal hyperexcitability associated with kindling. More controversial reports have suggested that TRH administration may be of therapeutic benefit for patients afflicted with schizophrenia. Trhr1 knockout mice have central hypothyroidism and mild hyperglycemia but exhibit normal growth and development as well as normal body weight and food intake. Behaviorally, Trhr1−/− mice display increased anxiety and depression.

Although Myelodysplasia/myeloid leukemia factor 1 gene (MLF1) did not show any relation to psychiatric disease, this gene has a role in the brain. Initially discovered to be involved in a chromosomal translocation associated with myelodysplastic syndrome and acute myeloid leukemia, it has been determined that MLF1 has protective effects on neuronal cell death in drosophila. In mice, Mlf1−/− embryos failed to develop beyond embryonic day 6.5.

| Locus | Overlapping ROH coordinates | Length (Kb) | Gene content | ASD patients |
|-------|----------------------------|------------|--------------|--------------|
| 8q23.1 | 110051909–110185293 | 820 | TRHR | F4-015, F6-024, F7-029, F9-038, F40-186 |
| 3q25.32 | 157813799–158331136 | 517 | MLF1, RSRC1, SHOX2 | F4-015, F6-024, F20-093, F27-122, F28-127, F36-169 |
| 2p22.1 | 38790327–39664219 | 873 | SOS1, MAP4K3, CDKL4, HNRPLL, SRSF7, DHX57, MORN2, ARHGEF33 | F1-001, F3-010, F6-024, F18-083, F19-88, F19-89, F29-133 |
| 18q12.1 | 29687013–30278521 | 591 | RNF138, WBP11P1, FAM59A, KLHL14, MEP1B | F3-010, F4-015, F20-093, F22-101, F28-127, F35-164, F36-169 |

Table 2. ROH regions found in at least 5 ASD patients. ROH chromosomal locations and coordinates for the overlapping regions are shown (human genome build hg19). Genes within the ROH are listed. ASD patients with the corresponding ROH are indicated (out of the 37 ASD patients in our cohort).
The CNTNAP2 protein, a member of the neurexin superfamily, has been repeatedly associated with a wide spectrum of neuropsychiatric disorders, such as developmental language disorders, autism, epilepsy, and schizophrenia. This cell adhesion molecule is crucial for the maintenance of functional synapses. A mouse knockout model created in 2007 shows features of autism and seizures. It has been suggested that CNTNAP2 common variants may represent susceptibility factors for other language-related deficits such as specific language impairments, in addition to autism. Heterozygous mutations in CNTNAP2 are frequently associated with susceptibility to autism. CNTNAP2 was also identified as an ASD causative gene due to its association to semantic-pragmatic skills and social inhibition. A deletion in CNTNAP2 leads to delayed myelination in the brain with abnormal T2 hyperintensities and causes loss of white matter volume. In addition, a homozygous frameshift mutation in CNTNAP2 was discovered in an Amish family with syndromic ASD (Cortical dysplasia focal epilepsy syndrome), a neuron migration disorder with epilepsy, language regression, intellectual disability, and ASD. In our study, CNTNAP2 encompasses nine additional ROH found in 7 ASD patients. Mutational screening of all ASD patients, however, did not reveal any previously reported mutation. We did observe a novel 5 bp insertion in the intron upstream of exon 23. Although we observed the alternate homozygous genotype more frequently in the group of affected individuals and their family members compared to the unaffected control group (28.3% versus 16%, respectively, or a difference of 12.3%), the difference in allele frequency was not statistically significant (p > 0.05) between the two groups. Results, therefore, do not support this insertion as a high-risk genetic variant for ASD but as a benign polymorphism commonly found in the Lebanese population.

Figure 1. Biological network created using pathway analysis to identify biological functions and disorders associated with genes selected from ROH regions common to at least 5 ASD patients in an overlapping manner. Thirteen genes are depicted in direct interactions. Cellular processes are highlighted in yellow, cellular processes related to the brain are in blue, and disorders are in purple.

Figure 2. The human CNTNAP2 locus at 7q35. Schematic indicating the 24 exons (blue bars) of CNTNAP2. Red lines indicate ROH regions found in 7 ASD patients. Note that there is no complete overlap of ROH regions between the 7 patients.
Figure 3. Biological network created using pathway analysis linking genes of interest DPP4, TRHR, MLF1, and CNTNAP2 to cellular processes related to the brain (in blue) and neurological disorders (in purple).

Figure 4. An insertion of 5 bp (chr7: 148,106,477 C > TTCTG) upstream of exon 23 (red arrow) of CNTNAP2. Representative Sanger sequence data depicting the 5 bp insertion. Intrinsic sequence is shown in black, coding sequence in blue, and the insertion in red.

Table 3. Genotype and allelic distribution of CNTNAP2 variant in ASD patients, their family members, and unaffected controls. N: reference allele; V: alternate allele with the TTCTG insertion at intron/exon junction of exon 23.
Conclusion
This study highlights the effectiveness of microarray technology and homozygosity mapping in the search for ASD susceptibility genes in the Lebanese population. We propose in this study that DPP4, TRHR, and MLF1 are 3 novel candidate genes for ASD. Mutational screening of CNTNAP2, which is classified as a strong autism risk gene, uncovered a novel CNTNAP2 5 bp intronic insertion upstream of exon 23 in Lebanese ASD cases and unaffected controls. Yet, one cannot exclude that this novel CNTNAP2 polymorphism we uncovered may contribute to the ASD phenotype, depending on a second hit in the genome and other modifying factors.

Methods
Patient Samples. Blood was collected from 36 families, with 37 ASD patients (35 males and 2 females), who fulfilled Diagnostic and Statistical Manual of Mental Disorders-4/5 criteria for autism (Table 1). A protocol was developed with consent forms that were approved by the Institutional Review Board (IRB) of the American University of Beirut (IRB protocol number: BIOCH.RB.06 Protocol Name: Autism susceptibility genes in the Lebanese population). All experimental protocols were carried out in accordance with the relevant guidelines and approved by the relevant committees. Informed consent was obtained from the parents for themselves, siblings if above the age of twelve, and on behalf of children with ASD and those below 12 years of age from the parents by the investigators who are all CITI certified. In addition, a control cohort of 100 unaffected participants was recruited. DNA samples were extracted from peripheral blood using QIAamp® blood midi kit (Qiagen, Inc., Valencia, CA).

Microarray Analysis. Affymetrix Cytogenetics Whole-Genome 2.7 M Microarrays and CytoScan™ HD arrays carry 2,700,000 unique, non-polymorphic probes covering the whole genome for detection of 35 kb or higher copy number variants as well as runs of homozygosity (ROH). Analysis was performed according to manufacturer protocols (Affymetrix Inc., Santa Clara, CA). Briefly, 3 μl of genomic DNA (33 ng/μl) are denatured and neutralized, and then amplified by PCR. The PCR products are then purified using magnetic beads (Beckman Coulter, Beverly, MA). The purified PCR products are then hybridized overnight to the arrays. The arrays are washed and stained using the GeneChip® Fluidics Station 450, and DAT images are acquired using the GeneChip® Scanner 3000 (Affymetrix Inc.). Microarray data are available in the ArrayExpress database (www.ebi.ac.uk/arrayexpress) under accession number E-MTAB-4963. Data analysis is performed using Affymetrix Chromosome Analysis Suite (CHAS).

Pathway Studio software version 9.0 (Ariadne Genomics, Rockville, Md., USA) was used to identify pathways and biological processes common to genes within ROH regions and place them in networks related to neuronal disease or autism.

PCR and Sanger Sequencing. Primer pairs listed in Table 4 were used to amplify coding exons of CNTNAP2, including intronic flanking regions, from genomic DNA with a standard polymerase chain reaction (PCR) over 35 cycles with a 56.7 °C annealing temperature. PCR reactions were performed using 100 ng of DNA, 50 ng of each primer, 1X IQ Phusion mix (Finnzymes®) containing buffer, dNTPs, and Taq polymerase. PCR products were purified on 1% agarose gel using the GE Healthcare® DNA purification kit. DNA was then screened for mutations by unidirectional direct sequencing using the BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Mutations were confirmed with an independent PCR and bidirectional sequencing.

| Exon       | Primer sequence         | Amplicon size (bp) |
|------------|-------------------------|--------------------|
| CNTNAP2_ex8F | tcacgaatctatgcctgc     | 524                |
| CNTNAP2_ex8R | aasaaccaatctgccgctgaac  | 440                |
| CNTNAP2_ex14F | agagattcttgggaatgg     | 479                |
| CNTNAP2_ex14R | tggcactactcccttct      | 350                |
| CNTNAP2_ex17F | tegaccttaggagccgctg   | 314                |
| CNTNAP2_ex17R | ggcccaacactctcctggg    | 462                |
| CNTNAP2_ex20F | aggagagaatggaggaatgt   | 366                |
| CNTNAP2_ex20R | ttatgcacttgaggaatgt    | 437                |

Table 4. Primer sequences for amplification and sequencing of CNTNAP2 coding exons.
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