Published in final edited form as:

Schizophr Res. 2015 October ; 168(0): 421–428. doi:10.1016/j.schres.2015.07.002.

RARE VARIANTS IN THE NEUROTROPHIN SIGNALING PATHWAY IMPLICATED IN SCHIZOPHRENIA RISK

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Abstract

Multiple lines of evidence corroborate impaired signaling pathways as relevant to the underpinnings of schizophrenia. There has been an interest in neurotrophins, since they are crucial mediators of neurodevelopment and in synaptic connectivity in the adult brain. Neurotrophins and their receptors demonstrate aberrant expression patterns in cortical areas for schizophrenia cases in comparison to control subjects. There is little known about the contribution of neurotrophin genes

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CONTRIBUTORS

We certify that authorship for all individuals listed on this manuscript was justified through participation in the following: conception and design (TMK, DM, MVC, ID, AH), data collection and management (all authors), analysis and interpretation of data (all authors), drafting of the manuscript (TMK, DM, MVC), revising the manuscript critically for important intellectual content (all authors), and final approval of the manuscript (all authors).

CONFLICT OF INTEREST

The authors declare no conflict of interest

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in psychiatric disorders. To begin to address this issue, we conducted high-coverage targeted exome capture in a subset of neurotrophin genes in 48 comprehensively characterized cases with schizophrenia-related psychosis. We herein report rare missense polymorphisms and novel missense mutations in neurotrophin receptor signaling pathway genes. Furthermore, we observed that several genes have a higher propensity to harbor missense coding variants than others. Based on this initial analysis we suggest that rare variants and missense mutations in neurotrophin genes might represent genetic contributions involved across psychiatric disorders.

Keywords

schizophrenia; sporadic; de novo; ARMS; Kidins220; rare variant; neurotrophin; exome sequencing

1. INTRODUCTION

Schizophrenia is a complex genetic disorder that affects roughly 7 in 1000 individuals worldwide (McGrath et al., 2003). Several different mechanisms have been shown to confer risk for schizophrenia. One genetic contribution to illness risk is copy number variations (CNV), which are detected in several neurodevelopmental and psychiatric conditions, such as autism spectrum disorder, bipolar disorder and schizophrenia (Doherty et al., 2012; King and Lord, 2011; Sullivan et al., 2012). Further support for shared genetic architecture among psychiatric diseases was demonstrated by a genome-wide association analysis, which found variations in calcium-channel activity genes, with obvious pleiotropic effects, across five different psychiatric disorders (Cross-Disorder Group of the Psychiatric Genomics, 2013).

Despite several comprehensive efforts to define overlapping genetic susceptibilities, the signaling pathways that are associated with many psychiatric dysfunctions have not been fully defined. Several coexisting hypotheses have been proposed. Recent large exome sequencing studies of the genomes of autism spectrum disorder and schizophrenia trios have revealed a number of *de novo* mutations (Dong et al., 2014; Fromer et al., 2014). These findings, accomplished in different populations and samples, converge to the conclusion that *de novo* mutations may play a significant role in the pathophysiology of these disorders. These studies also point to the enrichment of rare occurring *de novo* mutations in genes encoding for pre- and postsynaptic proteins. In addition to genetic contributions, schizophrenia etiology is significantly influenced by environmental factors, especially early life stress during pre- and postnatal life (Caspi and Moffitt, 2006; Fumagalli et al., 2007).

A very important family of proteins responsible for correct neurodevelopment and maintenance of functional neuronal networks are neurotrophins and their receptors. In particular, the neurotrophin brain-derived neurotrophic factor (BDNF) has been shown to be important for both the integrity and plasticity of neuronal connections in the central nervous system and is the best studied of all neurotrophins (Bekinschtein et al., 2014; Callaghan and Kelly, 2012; Park and Poo, 2013). Neurotrophins facilitate a variety of functions, including cell survival, differentiation, axonal and dendritic growth as well as synaptic plasticity, arborization and synapse formation (Chao, 2003; Park and Poo, 2013).

The actions of trophic factors entail two different transmembrane receptors, the Trk and the NGFR (p75) receptor (Chao, 2003; Huang and Reichardt, 2003). Upon binding of neurotrophins to their corresponding Trk receptors, adapter molecules such as Src homology 2-containing protein (Shc) and fibroblast growth factor receptor substrate 2 (FRS2) are recruited to specific docking sites within the Trk receptors (Zampieri and Chao, 2006). Neurotrophin binding to Trk receptors activates multiple intracellular pathways, including MAPK, PI3K-AKT and PLC γ -PKC, whereas p75 can engage the apoptotic machinery and induce morphological changes, such as growth cone arrest (Deinhardt et al., 2011; Gehler et al., 2004). Activation of any of the three before mentioned signaling pathways upon Trk receptor activation influences synaptic plasticity in a specific manner. MAPK signaling preferably acts on neuronal differentiation (Nakamura et al., 1996; Ortega and Alcantara, 2010). The Akt and PLC γ pathways are mainly activation is responsible for neuronal survival (Chao, 2003; Minichiello et al., 1999). Ankyrin repeat-rich membrane spanning protein (ARMS) or Kidins220 (Kinase D-Interacting Substrate Of 220 KDa) is a scaffold protein (Iglesias et al., 2000; Kong et al., 2001). Trio interacts with the neurotrophin receptor p75 as well as ARMS/Kidins220 at the plasma membrane (Deinhardt et al., 2011) and activates the MAPK signaling pathway and Rac1 (Bellanger et al., 1998), which are necessary for growth cone maintenance in dendrites (Deinhardt et al., 2011).

Gene expression studies on neurotrophin genes indicated decreased expression patterns in human brain. Altered BDNF expression is described in *post-mortem* cortices of schizophrenia cases (Issa et al., 2010; Ray et al., 2014; Weickert et al., 2003). Both BDNF and NT-3 protein expression levels were increased in the cortical areas and decreased in the hippocampus. (Durany et al., 2001) Another factor that points to the importance of neurotrophic factors like BDNF and NGF and their signaling pathways is their occurrence in brain regions that are found impaired in cases diagnosed with neurodegenerative and psychiatric conditions such as the cortex, hippocampus and pituitary gland (Goedert et al., 1986; Hefti and Weiner, 1986; Maisonpierre et al., 1991; Mariga et al., 2015).

Several genetic association studies and reports have elucidated the potential impact of the TrkB – BDNF system on the etiology of neurodevelopmental and psychiatric disorders. A mutation in the tyrosine kinase domain of TrkB (Y722C) was identified in a young male case with extreme obesity and developmental delay. The mutation decreases the autophosphorylation ability of TrkB and thus especially MAPK signaling (Yeo et al., 2004). Further corroboration of the importance of the neurotrophin signaling pathway for correct neurodevelopment is provided by a clinical report about four patients, each diagnosed with a variable degree of developmental delay and obesity with a deletion encompassing the BDNF gene (Shinawi et al., 2011). Most studies on BDNF have emphasized a SNP in the prodomain Val66Met (rs6265), with ambiguous results among psychiatric disorders and related endophenotypes (Jiang et al., 2005; Schumacher et al., 2005; Soliman et al., 2010; Tsai et al., 2003; Verhagen et al., 2010; Zou et al., 2010). An interaction of BDNF and TrkB SNPs was found to be involved in the susceptibility of paranoid schizophrenia in Han Chinese, although single SNPs in these genes did not confirm this finding (Lin et al., 2013).

Based on the neurodevelopmental impact of neurotrophin signaling pathways on neuronal networks and the ambiguity of the current findings on neurotrophins and schizophrenia

etiology, we performed targeted exome capture of selected neurotrophin genes in 48 schizophrenia related psychosis cases. The authors are aware there can be limitations in studying a small sample size. However, the strength of this study is the deep coverage of the target genes (>50x for 99% of exon sequence) and the ability to assess a well-defined pathway of interacting genes. The detection of ultra rare genetic variants and *de novo* mutations support the neurotrophin signaling pathway as a potential contributor to schizophrenia etiology.

2. MATERIAL AND METHODS

Ascertainment and characterization of cases

The study subjects were recruited from Bellevue Hospital Center and New York University and IRB approvals were obtained from both institutions. Cases with schizophrenia or schizoaffective disorder, age 18–55 years, were recruited from treatment settings and all provided informed consent for the study. Diagnosis was determined by best estimate diagnostic procedures that included the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al., 1994) conducted by reliable master's level diagnosticians and family history assessed with the Family Interview for Genetic Study (FIGS) (Maxwell, 1992). The inter-rater reliability was $\kappa=.95$ for DSM-IV diagnosis and $\kappa=.80$ for individual symptoms. Symptoms were assessed with the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) and expressed as factors for the severity of positive, negative, dysthymia, activation and autism symptoms (White et al., 1997). The Wechsler Adult Intelligence Scale (WAIS-III) (Wechsler, 1999) was used to assess Verbal, Performance, and Full Scale IQ. Early trauma exposure was assessed using the Early Trauma Inventory (ETI) (Bremner et al., 2000).

Sample size and DNA source

Targeted exome capture of 48 non-related schizophrenia-affected individuals of diverse ethnicity was conducted on DNA derived from peripheral leukocytes.

Targeted exome sequencing and variant call

Genomic DNA was isolated from leukocytes derived from whole blood using the using a simple salting out procedure (Miller et al., 1988). The DNA (500 ng) was sheared to an average of 150 bp. Barcoded libraries were prepared using the Kapa Low-Throughput Library Preparation Kit Standard Libraries and amplified using the KAPA HiFi Library Amplification kit (Kapa Biosystems) (8 cycles). Quantification was performed using Qubit Fluorimetric Quantitation (Invitrogen) and Agilent Bioanalyzer. An equimolar pool of the 4 barcoded libraries (300 ng each) was used as input to exon capture using one reaction tube of the custom Nimblegen SeqCap EZ (Roche) with custom probes targeting the coding exons of the genes of interest. Capture by hybridization was performed according to the manufacturer's protocols with following modifications: 1 nmol of a pool of blocker oligonucleotides and post-capture PCR amplification was done using the KAPA HiFi Library Amplification kit in a 60 ul volume. Pooled capture library was quantified by Qubit (Invitrogen) and Bioanalyzer (Agilent) and sequenced in on an Illumina MiSeq sequencer using the 2×150 paired-end cycle protocol, or on an Illumina HiSeq 2500 using a 2 × 100

run. Reads were aligned to the hg19 build of the human genome using BWA with duplicate removal using samtools as implemented by the Illumina MiSeq Reporter. Variant detection was performed using GATK UnifiedGenotyper. Variants were annotated with Charity annotator to cross-reference against known dbSNP, 1000 Genomes, ESP6500/COSMIC mutations and Schizophrenia Exome Sequencing Genebook entries.

3. RESULTS

Neurotrophin signaling pathway genes harbor rare missense coding mutations

The goal was the detection of rare missense coding polymorphisms and novel missense variants in the Trk receptors (TrkA, TrkB, TrkC), p75 receptor, the neurotrophins NGF, BDNF, NT-3 and NT-4 and the Trk interactors ARMS/Kidins220 and TRIO. Proteins encoded by this gene family have been well characterized in their ligand-receptor specificity and downstream signaling. Notably, 37 of 48 cases harbored rare missense coding polymorphisms (MAF $\leq$ 1%) and novel missense coding variants in at least one of the above-mentioned genes (Table 1).

Cases display lower IQs and depressive episodes

The clinical cases that were examined exhibited psychotic symptoms and shared other comorbidities, such as joint problems and depressive episodes. More than 80% of the cases harboring missense polymorphisms and novel variants in the neurotrophin and interacting genes displayed significantly lower verbal IQ levels (62–90) and one third of them were had learning disabilities (Table 2). Seven of 48 cases (14%) showed novel variants in one gene (NTRK1, NGFR, TRIO, ARMS/Kidins220) of the neurotrophin signaling pathway. One of these variants occurred in two cases independently (NTRK1, G4S) and is located in its signal peptide. A novel variant in NTRK3 (TrkC) in one case (I525F) is located between the SHC binding site (Y516) and the tyrosine kinase domain (AA 538–839). Another case with severe paranoid delusions and adolescent onset harbored a novel missense coding variant (H1085R) in the proline-rich domain of ARMS/Kidins220, a scaffold protein and a substrate of Trk receptors (Iglesias et al., 2000; Kong et al., 2001). Two cases demonstrated novel variants in the Rho-GEF TRIO: one harbors a novel variant in its kinase domain (V2941I); a further one a novel variant in the IG-like C2 type domain (T2725I). In different case, a novel missense variant occurred in the NGFR (p75) receptor gene (G7R) in its signal peptide. No novel missense coding variants were detected in NTRK2 (TrkB), or the ligands BDNF, neurotrophin-3 (NT-3) or neurotrophin-4 (NT-4).

NTRK1-ARMS/Kidins220-TRIO genes harbor more than 50% of rare variants

In 17 cases, 23 rare missense coding polymorphisms (MAF 0.05 – 1%) were discovered, which had been described in the 1000 Genomes Project. More than 50% of rare missense coding polymorphisms was observed in ARMS/Kidins220, TRIO and NGF. In eight cases, rare missense coding polymorphisms occurred in NTRK1. The BDNF prodomain single nucleotide polymorphism Val66Met (rs6265) was observed in eight cases, of which seven were heterozygous (Val/Met) and one was homozygous (Met/Met). One common SNP in the NGF prodomain (rs6330, Ala35Val) was observed in 24 of 37 cases.

NTRK1-ARMS/Kidins220-TRIO genes reveal highest genetic burden

A genetic burden analysis was performed on the neurotrophin genes. In this way the probability of large genes to harbor more missense variants than in small genes is taken into consideration (Table 4). The top half of the table mainly represents the NGF pathway, with the NGF receptor NTRK1, the p75 (NGFR) receptor, the NGF gene and the NGF receptor scaffold ARMS/Kidins220. The highest burden was exhibited by the NTRK1-ARMS/Kidins220 pathway with more than 50% of all observed rare neurotrophin gene polymorphisms. The highest amount of variation in a single gene was observed in the Rho-GEF Trio with 11 different variants across the sample. The NGF receptor NTRK1 revealed the highest single genetic burden in our dataset with seven different missense variants across the complete sample and is followed by the NGFR receptor with two variants.

ARMS/Kidins220 displays several rare variants in protein interaction domains

Of particular interest are the findings of five variant carriers in the scaffold protein ARMS/Kidins220 (Table 2), since mutations in this gene have not been reported to be linked before to psychiatric disorders. The subject carrying ARMS/Kidins220 H1085R is a particular case associated with a life-long history of psychiatric problems. The three A1299G variant carriers reported auditory, bizarre visual hallucinations and psychomotor retardation. The variant carrier A557V reported auditory, visual and olfactory hallucinations in combination with manic episodes.

The novel mutation (H1085R) in ARMS/Kidins220 is located in sustained MAPK signaling initiation region

The ARMS/Kidins220 gene is a scaffold protein and contains multiple protein interaction domains (Figure 1). It is rapidly tyrosine phosphorylated by all three Trk receptors after neurotrophin binding. Two of the 48 cases demonstrated rare variants (MAF < 0.01) in the coding sequences of ARMS/Kidins220. Both variants entail amino acid exchanges (A1299G) and (A557V) and are located between TM2 and 3 as well as the SAM domain of ARMS/Kidins220. The third variant in the proline-rich domain (H1085R) is not annotated in any accessible genomic database. The heterozygous mutation of H1085R is located in the proline-rich domain of ARMS/Kidins220 (Figure 1). A single tyrosine phosphorylation site (Y1096) in this domain is responsible for the recruitment of the Crk-like protein (CrkL), which contributes to sustained MAPK signaling and initiates neurite outgrowth (Arevalo et al., 2004). All carriers of the rare variant and the private mutation are heterozygous (Table 3). The other ultra-rare variant A1299G is located in the sterile alpha motif (SAM) domain. The SAM domain is responsible for self-association of proteins and also enables the binding to non-SAM proteins (Kim and Bowie, 2003; Schultz et al., 1997).

The majority of rare variants are located in functional or protein interaction domains

The results from this study suggest that rare polymorphisms and novel mutations occur in protein interaction domains of Trk receptor genes and their interaction partners. Two genes stood out among those sequenced in the sample: the Rho-GEF Trio and the scaffold protein ARMS/Kidins220. TRIO revealed most rare polymorphisms and one novel mutation in its C-terminal regions, especially in its kinase domain, which is known to interact with the p75

receptor (Deinhardt et al., 2011). The rare polymorphisms and novel mutation found in ARMS/Kidins220 are all located in important protein interaction domains. This observation indicates that proteins representing the Trk receptor signaling pathway may contribute to schizophrenia-related symptoms.

4. DISCUSSION

In this study, 37 of 48 cases with schizophrenia-related psychosis harbored missense coding polymorphisms and novel variants in neurotrophin genes. The NGF-NTRK1-ARMS/Kidins220 pathway harbored more than 50% of all observed missense coding variants among the cases. Interestingly, novel missense coding variants were not detected in NTRK2 (TrkB), BDNF, neurotrophin-3 (NT-3) or neurotrophin-4 (NT-4). This observation suggests that mutations disrupting neurotrophin genes are rare and subjected to high selection pressure and enormous conservation across species. The finding that neurotrophin genes reveal an extremely low *de novo* mutational burden is corroborated by previous large scale whole exome sequencing studies. Only two mutations in BDNF have been described, which occurred in two schizophrenia cases (G198D and G190V). Another mutation in NT-4 (D162Y) was observed in one case. No variants were found in NGF or NT-3 in two studies, comprising of 2,536 schizophrenia cases and 2,543 controls as well as 623 parent-child trios (Fromer et al., 2014; Purcell et al., 2014).

The accumulation of rare genetic variants in the NGF-NTRK1-ARMS/Kidins220-TRIO pathway is striking. Multiple genetic hits in genes forming a functional pathway might promote pathophysiological changes in the CNS associated with schizophrenia. A previous study of the NGF prodomain detected a SNP at a high frequency (A35V), which is a susceptibility factor for mild cognitive impairment (Di Maria et al., 2012). NTRK1 activation upon NGF binding is crucial for the maintenance of neurons in the CNS and in the periphery. Variants in the signaling peptide might effect receptor trafficking from trans-Golgi network to the plasma membrane. Once NTRK1 is activated, its immediate downstream interaction partner, the scaffold protein ARMS/Kidins220, becomes phosphorylated (Arevalo et al., 2004). ARMS/Kidins220 consists of several protein interaction domains with known and unknown functions. The N terminal part consists of 11 Ankyrin repeats, which facilitate the interaction with the soluble Rho guanine nucleotide exchange factor TRIO. It is involved in growth cone maintenance and stability (Deinhardt et al., 2011) and interacts with ARMS/Kidins220 through the ankyrin repeats (Neubrand et al., 2010b). The ankyrin repeats are followed by four transmembrane domains (TM), a polyproline stretch, a sterile alpha motif (SAM) domain and a kinesin light chain (KIM) domain (Figure 1). All variants detected in this study are located either in the intracellular loop between the transmembrane domains 2 and 3 or in C terminal protein interaction domains. Previously it was found that the TM domains are necessary and sufficient for an interaction with the AMPA receptor subunit GluA1 (Arevalo et al., 2010). In three cases, the variant A1299G was identified, which is located in the SAM domain. The SAM domain is found in a variety of proteins to facilitate homo- and heterodimerization (Kim and Bowie, 2003). The mutation in the polyproline stretch (H1085R) has not been reported before. Previous studies confirmed that the polypro line stretch of ARMS/Kidins220 interacts specifically with the CrkL adaptor, which induces sustained MAPK signaling and is relevant

to synaptic plasticity (Arevalo et al., 2004). Here, no genetic variants were identified in the KIM domain, which enables the intracellular trafficking of ARMS/Kidins220 by kinesin-1 (Bracale et al., 2007).

These protein interaction domains of ARMS/Kidins220 are similar to other proteins, such as SHANK3, a candidate gene for autism and schizophrenia. SHANK3 is a scaffold protein that interacts with the cytoskeleton and also with PSD-95 and glutamate receptors (Gauthier et al., 2010; Gauthier et al., 2009). ARMS/Kidins220 shows high structural and functional similarity with the SHANK family, thus it is possible that ARMS/Kidins220 might also represent a susceptibility gene for schizophrenia. Their similarity is highlighted by interactions of both proteins with AMPA and NMDA receptors (Arons et al., 2012; López-Menéndez et al., 2009) and similar intracellular localization (Wang et al., 2011; Wu et al., 2009).

The other member of this signaling pathway is the Rho-GEF TRIO. It promotes axon guidance and neurite outgrowth through interaction with ARMS/Kidins220 (Neubrand et al., 2010a). Rare variants detected in TRIO accumulate in its kinase domain. TRIO forms a ternary complex through its kinase domain with the neurotrophin receptor p75 and SorCS2 and keeps growth cones stable (Deinhardt et al., 2011). Upon dissociation of p75 from TRIO, growth cone collapse occurs through downstream Rac1 inactivation. Thus, variants in the kinase domain might affect the affinity of p75 with TRIO and this way immediately influence growth cone stability at dendrites.

Recently, a study on *post-mortem* brain tissue of bipolar disorder and schizophrenia cases revealed that spine density in the dorsolateral prefrontal cortex is significantly reduced in comparison to controls (Konopaske et al., 2014; Penzes et al., 2011). Dendritic spine changes have been proposed in neuropsychiatric disorders (Glausier and Lewis, 2013; Penzes et al., 2011). Morphological changes in dendritic complexity are dependent upon Trk receptor activity and multiple interaction partners (Chen et al., 2012; Marler et al., 2008; Wu et al., 2009). Changes in neuronal morphology and synaptic function are consistent with the hypothesis that schizophrenia is the reflection and summation of many multilayered genetic variations and signaling pathways (Arnedo et al., 2014; Fromer et al., 2014).

CONCLUSION

This study suggests that neurotrophin signaling genes represent a potential susceptibility signaling pathway for schizophrenia-related psychosis. Neurotrophins and their Trk receptors are involved in a variety of neuronal functions, including synaptic transmission and signal transmission as well as cardiovascular development. This study provides evidence that accumulation of rare variants in a functional genetic network might influence the pathophysiology of schizophrenia. Focusing on genetic vulnerability in genes that form functional networks with known mechanism and action might offer a promising starting point for further translational research in order to identify disease-relevant mechanisms. Further studies with an extended sample size as well as biochemical assays of the contributing pathways including the novel variants will assess the precise contribution of the

neurotrophin signaling pathway as a modulator of synaptic function and its effects upon mood and psychiatric disorders.

Acknowledgments

ROLE OF FUNDING SOURCE

This work was supported in part by National Institutes of Health Grants (DM), RC1-MH088843 (DM), 5K24MH001699 (DM), NYU CTSI UL1TR000038 (DM), and MH086651 (MVC).

This work was supported in part by National Institute of Health (NIH) Grants R01-MH59114, RC1-MH088843 and 5K24MH001699, NYU CTSI UL1TR000038 (to D.M.) and NIH grants MH086651 and NS21072 (to M.V.C.). The authors wish to acknowledge Karen Rothman, BA for her assistance in preparing this manuscript.

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Figure 1.

Location of rare variants and mutation in ARMS/Kidins220 and interaction partners. Two rare variants (star, Ala557Val and Ala1299Glu) and one mutation (hexagon, His1085Arg) were detected in 5 of 48 cases.

Table 1

Cases with rare missense coding polymorphisms and novel variants in neurotrophin genes

Case ID	Gene	Category	Genotype	rs number	1000 Genomes GMAF	Effect	PANSS (positive:negative:general psychopathology)
1	NTRK1	missense Pol	G/A	rs79678945	0.09%	R85H	x
	NGF	missense Pol	G/A	rs6330	31%	A35V	
2	NTRK1	novel variant	G/A	x	x	G4S	14--8--22
		missense Pol	G/A	rs56320207	1%	R438Q	
3	NGF	missense Pol	G/A	rs6330	31%	A35V	14--16--29
4	NGF	missense Pol	G/A	rs6330	31%	A35V	18--15--26
5	NGF	missense Pol	G/A	rs6330	31%	A35V	x
	BDNF	missense Pol	C/T	rs6265	23%	V66M	
6	NGF	missense Pol	A/A	rs6330	31%	A35V	18--7--22
7	NGF	missense Pol	G/A	rs6330	31%	A35V	20--17--33
	TRIO	missense Pol	A/G	rs73749289	0.32%	K2816R	
8	NGF	missense Pol	G/A	rs6330	31%	A35V	13--7--20
9	NGF	missense Pol	G/A	rs6330	31%	A35V	19--14--45
	ARMS/KIDINS220	missense Pol	G/C	rs76164009	0.27%	A1299G	
10	NTRK3	novel variant	T/A	x	x	I525F	x
	BDNF	missense Pol	C/T	rs6265	23%	V66M	
11	NTRK1	missense Pol	C/T	rs55909005	1%	T237M	19--14--26
		missense Pol	G/A	rs62640939	0.18%	A752T	
		novel variant	T/C	x	x	H1085R	
12	NTRK1	missense Pol	C/T	rs201472270	x	R6W	25--22--44
	BDNF	missense Pol	C/T	rs6265	23%	V66M	
	NGF	missense Pol	G/A	rs6330	31%	A35V	
13	NGF	missense Pol	G/A	rs6330	31%	A35V	15--13--32
	TRIO	missense Pol	C/T	rs61740945	0.23%	R3083C	
	BDNF	missense Pol	C/T	rs6265	23%	V66M	
14	NGF	missense Pol	G/A	rs6330	31%	A35V	9--7--23
	NGF	missense Pol	G/A	rs6330	31%	A35V	
15	NGF	missense Pol	G/A	rs6330	31%	A35V	21--19--35

Case ID	Gene	Category	Genotype	rs number	1000 Genomes GMAF	Effect	PANSS (positive:negative:general psychopathology)
16	NGF	missense Pol	G/A	rs6330	31%	A35V	10--22--32
17	NGF	missense Pol	G/A	rs6330	31%	A35V	9--18--38
18	ARMS/KIDINS220	missense Pol	G/C	rs76164009	0.27%	A1299G	22--15--39
19	BDNF	missense Pol	T/T	rs6265	23%	V66M	11--10--36
	NGF	missense Pol	G/A	rs6330	31%	A35V	
20	NGF	missense Pol	G/A	rs6330	31%	A35V	x
	NTRK1	missense Pol	C/T	rs55909005	1%	T237M	
21	NGF	missense Pol	G/A	rs6330	31%	A35V	22--24--46
	TRIO	missense Pol	G/A	rs16903474	1%	A1613T	
		missense Pol	C/T	rs115054458	1%	A2464V	
22	NGF	missense Pol	C/T	rs11466110	1%	V72M	
		missense Pol	G/A	rs6330	31%	A35V	x
	TRIO	novel variant	G/A	x	x	V2941I	
	ARMS/KIDINS220	missense Pol	G/C	rs76164009	0.27%	A1299G	
23	BDNF	missense Pol	C/T	rs6265	23%	V66M	x
	TRIO	missense Pol	A/T	rs55900671	1%	K3037M	
24	BDNF	missense Pol	C/T	rs6265	23%	V66M	9--23--28
	NGF	missense Pol	G/A	rs6330	31%	A35V	
25	ARMS/KIDINS220	missense Pol	G/A	rs201557833	0.05%	A557V	x
26	BDNF	missense Pol	C/T	rs6265	23%	V66M	x
		missense Pol	G/A	rs6330	31%	A35V	
27	NGF	missense Pol	C/T	rs11466110	1%	V72M	x
		missense Pol	G/A	rs6330	31%	A35V	
28	TRIO	missense Pol	G/A	rs16903474	1%	A1613T	x
29	NTRK1	missense Pol	C/T	rs55909005	1%	T237M	12--21--28
30	NGF	missense Pol	G/A	rs6330	31%	A35V	x
31	TRIO	novel variant	C/T	x	x	T2725I	28--18--42
32	NGF	missense Pol	G/A	rs6330	31%	A35V	15--9--20
33	NTRK1	novel variant	G/A	x	x	G4S	15--16--26

Case ID	Gene	Category	Genotype	rs number	1000 Genomes GMAF	Effect	PANSS (positive:negative:general psychopathology)
	NGF	missense Pol	A/A	rs56320207	1%	R438Q	
		missense Pol	G/A	rs6330	31%	A35V	
		missense Pol	G/A	rs16903474	1%	A1613T	
	TRIO	missense Pol	A/G	rs73749289	0.32%	K2816R	
34	NGFR	novel variant	G/C	x	x	G7R	17--9--28
35	NTRK1	missense Pol	G/A	rs56320207	1%	R438Q	17--14--32
	NTRK3	missense Pol	C/A	rs146792958	x	Q389H	
36	NTRK1	missense Pol	C/T	rs55909005	1%	T237M	18--12--37
37	NGF	missense Pol	G/A	rs6330	31%	A35V	9--11--27

Clinical features of cases harboring rare polymorphisms and novel mutations in neurotrophin receptor pathway genes

Table 2

ID	Age	Sex (1=m; 2=f)	Onset	VIQ	PIQ	Odor ABSOTHRES	Odor UPSI/TOT	PANSSNEG	PANSSPOS	PANSSACT	PANSSDYS	PANSSAUT	ETI	Learning disabilities Yes/No (1/0)	Notes	Onset of depression	YMANTOT
1	48	1	25	79	72	2.52	11						5	0		38	0
2	37	2	15	84	64	3.95	31	11	9	8	5	6	18	0		15	20
3	29	2	21	95	74	4	37	19	12	8	15	11	3	0		15	0
4	48	1	25	98	111	4.19	36	16	9	12	13	11	6	1	hyperactive	16	15
5	26	1	18											1		N/A	15
6	23	1	18	130	121	2	21	10	14	10	6	8	4	1	time management	N/A	15
7	34	1	23	90	92	4	33	19	15	8	13	12		0	1	N/A	4
8	44	1	18			3.25	38	10	9	8	9	6	2	0		N/A	5
9	52	1	20	86	72	4.88	34	16	13	14	19	15	9	1	special education	27	6
10													6	0	0	36	4
11	50	1	26	75	89	3.13	24	12	15	8	12	12	25	0	0	N/A	10
12	52	1	22	79	73	2.13	35	25	16	16	19	13	5	1	mild dyslexia	23	20
13	55	2	22	76	84	3.19	25	17	10	9	6	14	14	0		N/A	5.5
14	49	2	18	111	109	3.94	39	10	7	8	12	6	12	0		N/A	2
15	51	1	20	103	84		20	20	14	12	13	9	18	0		N/A	10
16	52	2	16	72	75	3.75	32	24	11	8	13	15	2	0		16	1
17	41	1	11	66	64	3.31	36	19	8	8	19	11	9	1	special education	N/A	1
18	45	1	21	84	74	2	10	20	16	10	17	15	17	1		N/A	17
19	47	1	14	79	78	3.88	32	12	9	10	22	9	21	0		13	3
20														1	ADHD in college	18	N/A
21	39	1	14	71	79	3.19	34	23	15	11	21	12	15	1	hyperactive	16	23
22													2	1	dyssynergic	18	N/A
23	48	2	16	75	83	3.25	36						6	0		29	21
24	52	2	30	77	77	4.06	32						4	0		31	0
25														0			N/A

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ID	Age	Sex (1=m; 2=f)	Onset	VIQ	PIQ	Odor ABSOTHR	Odor UPSITOT	PANSSNEG	PANSSPOS	PANSSACT	PANSSDYS	PANSSAUT	ETI	Learning disabilities Yes/No (1/0)	Notes	Onset of depression	YMANTOT
26																	N/A
27														1	special education	N/A	N/A
28														0		24	N/A
29	46	1	25	63	62	2	20	23	7	6	5	14	14	0		N/A	9
30	49	1												1	hyperactive	9	N/A
31													20	0		8	21
32	52	2	23	88	72	3.06	34	12	10	6	9	9	11	0		14	8
33	51	1	26	81	78			18	10	6	10	7	1	1	dyslexia	N/A	8
34	43	2	16					12	13	6	15	7	15	0		32	8
35	47	1	39	88	87			14	16	9	19	11	8	0		37	12
36	25	1	16	105	104			15	11	12	21	8	7	0		19	20
37	45	1	31	62	94			14	7	8	12	6	2	0		21	4

Table 3

Localization of ARMS/Kidins220 rare variants and novel mutation

ID	Exon	Substitution	Domain	Identifier	Class
A	29	A1299G	SAM	rs76164009	Rare SNP
B					
C					
D	15	A557V	between TM 2 and TM 3	rs201557833	MUTATION
E	24	H1085R	Proline-rich	x	

Table 4

Mutational burden of neurotrophin genes and interaction partners (missense coding variants)

Gene	Genetic burden	Gene length (bp)	Total Counts	Variables
NTRK1	10.57	66,211	16	7
NGFR	10.14	19,728	3	2
NGF	3.82	52,312	30	2
KIDINS220	3.56	112,353	24	4
TRIO	2.83	388,425	14	11
BDNF	1.49	67,166	8	1
NT-3	1.12	89,425	14	1
NTRK3	0.52	381,770	2	2