Hereditary Genetics

Research Article   **Open Access**

A Single Nucleotide Polymorphism in the *ARHGEF6* Gene is Associated with Increased Risk for Autism Spectrum Disorder

Matthew Pratt-Hyatt[1]*, Rebecca A Haeusler[2], Kobe C Yuen[3] and William Shaw[1]

[1]*The Great Plains Laboratory, Lenexa, Kansas, USA*
[2]*Department of Pathology and Cell Biology, Columbia University, New York, USA*
[3]*Stowers Institute for Medical Research, Kansas City, USA*

Abstract

Background: Autism Spectrum Disorder (ASD) comprises a range of neurological conditions that can be a lifelong developmental disability. ASD is characterized by deficits in social communication and interaction with repetitive patterns of behavior and interests.

Methods: The study population consisted of 155 ASD subjects (134 males, 21 females) selected randomly from Great Plains Laboratory subjects (84 males and 21 females) as well as from the Autism Genetic Resource Exchange (50 male subjects). Subjects were diagnosed by the CARS, DSM-IV, ABC, ADI-R, ADOS, PL-ADOS, or the BSE criteria for pervasive developmental ailment and Childhood Autism Rating Scale. A total of 247 individuals were used as controls (106 males, 141 females).

Results: In our study we identified a single nucleotide polymorphism (SNP), rs2295868 in the *ARHGEF6* gene (Rac/Cdc42 Guanine Nucleotide Exchange Factor 6) that was present in 36% of patients with ASD *vs.* 9% of controls. *ARHGEF6*, a Rho GTPase, is expressed mainly in the brain, immune system, and intestines.

Conclusion: SNP rs2295868 on the *ARHGEF6* gene has significant association with ASD (odds ratio 4.09, *p*=2.31 × 10^{-4}).

Keywords: Autism spectrum disorder; Single nucleotide polymorphism

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder with a highly heritable complex biological basis. ASD is characterized by impaired social interaction, communication, and repetitive behavior [1,2]. Many studies have concluded that ASD traits are highly heritable indicating a genetic link [2-4]. Twin studies support the genetic hypothesis of autism. Monozygotic twin concordance rate has been measured to be between 60 and 90% and a dizygotic concordance rate of approximately 5% [5,6]. ASD occurs in 1:68 individuals; however there are differences between the sexes. Approximately one in 42 boys (0.0238) and one in 189 girls (0.00529) has been identified as having ASD, which gives a male to female ratio of 4.5/1 [7,8]. Multiple studies have focused on the X chromosome to help explain this discrepancy between male and female rates of ASD [9-11]. The ratio of brain development and cognition genes is higher on the X chromosome than other chromosomes [12,13]. ASD is most likely not a monogenetic disorder, instead it is most likely a neurological condition that results from multiple mutations of several genes in multiple pathways. However, finding the common risk alleles that are found in a large population of autistic individuals can help us understand the potentially vulnerable pathways in neurons in order to develop more efficient interventions for ASD.

Studies have focused on finding candidate genes that might lead to susceptibility to develop ASD. Many of these candidate genes implicated in ASD are involved in neurodevelopmental pathways, transcriptional control, and hormones [14-16]. Changes in neurite branching and dendritic spine morphology, including size, shape, and number, are hallmarks of many neurological conditions, including ASD.

This study was performed with 247 controls (106 male and 141 female) and 155 patients on the autistic spectrum (134 male and 21 female). Among controls, ethnicities were 62% Caucasian, 20% East Asian, and 19% Hispanic. Among cases, ethnicities were 57% Caucasian, 23% East Asian, and 13% Hispanic.

Materials and Methods

Ethics statement

This study was approved by the ethics committee of WIRB. Written informed consent was obtained from all individuals and procedures had approval from institutional review board. The study protocol was performed in accordance with the Declaration of Helsinki.

Study population

The study population consisted of 155 ASD subjects (134 males, 21 females) selected randomly from Great Plains Laboratory subjects (84 males and 21 females) as well as from the Autism Genetic Resource Exchange (50 male subjects). Subjects were diagnosed by the CARS, DSM-IV, ABC, ADI-R, ADOS, PL-ADOS, or the BSE criteria for pervasive developmental ailment and Childhood Autism Rating Scale. A total of 247 individuals were used as controls (106 males, 141 females).

Genotyping

Genomic DNA was extracted from venous blood samples and saliva using the Agencourt Genfind V2 kit (Beckman Coulter, Indianapolis, IN, USA). The genomic DNA samples were captured with an inversion probe method for the SNPs in our panel (appendix A). These captured targets were sequenced on Illumina MiSeq sequencing system with 100 bp paired-end reads. Sequence results were mapped to UCSC hg19

*Corresponding author: Matthew Pratt-Hyatt, The Great Plains Laboratory, Lenexa, Kansas, USA, Tel: +913-341-6207; E-mail: pratthyatt@gmail.com

genomic reference using the ZiPhyr bioinformatics pipeline. All 247 individuals were genotyped for rs661426 and rs2295868. For rs616718, genotyping data was collected for 190 males (106 controls and 84 cases), and all 141 females.

Statistical analysis 1

All analyses were carried out using the statistical software R (Vienna Austria) [17]. SNPs were analyzed for association with ASD by logistic regression, individually and in an additive model. The logistic regression model was fitted stepwise both forward and backward. The receiver operating characteristic was analyzed to evaluate the predictive capacity of the model. To compare our minor allele frequencies to those in the 1000 Genomes project, we used dbSNP. To assess linkage disequilibrium in the 1000 genomes project, we used LDlink [18]. To assess linkage of the minor alleles in our population, and to assess the OR for ASD in subjects discordant for rs2295868 and rs661426, we performed chi-square tests.

Enhancer binding

Analysis of published ChIP-seq data was described previously [19]. Briefly, ChIP-seq data of active enhancer markers H3K4me1, H3K27ac and p300, together with CTCF, RAD21, YY1 and the RNA-seq data were mapped to the UCSC hg19 human genome by Bowtie2 aligner 2.1.0, allowing uniquely mapped reads only up to two mismatches. Individual mapped data was converted to bigwig files before being uploaded to the IGV Genome Browser for visualization. Active enhancer regions were marked by the co-localization of H3K4me1, H3K27ac and p300.

Results

Statistical analysis 2

Three SNPS from the ARHGEF6 gene (Rac/Cdc42 Guanine Nucleotide Exchange Factor 6) were analyzed because of their possible link to ASD. These SNPs (rs2295868, rs661426, and rs616718) were located in the *ARHGEF6* gene region (Figure 1), these were subjected to statistical analysis. One other *ARHGEF6* SNP rs545490 was excluded due to insufficient genotyping data. We noted that in our population, the minor allele frequencies differed from those observed in the 1000 genomes study: In our male control subjects, rs2295868 was less frequent and rs661426 was more frequent compared to 1000 genomes [20]. See Table 1 for full data.

Logistic regression of data

In individual analysis, two SNPs were significantly associated with ASD (Table 1): rs2295868 (p=2.31 × 10^{-5}, OR=4.09) and rs661426 (p=0.03, OR=1.77). We next performed stepwise fitting of a logistic regression model, using both SNPs as potential variables; both forward and backward fitting suggested that only rs2295868 predicted autism. In receiver operating characteristic analysis for rs2295868, the area under the curve (AUC) was 0.602. In females, none of the three SNPs were significantly associated with ASD (Table 1).

The loci of rs2295868 and rs661426 are 62 kilobases apart. Using the LDlink tool [18], we found that the two minor alleles are not in linkage disequilibrium in the 1000 Genomes population. In contrast, our own contingency table analysis showed tight association of the two minor alleles (p<0.0001). We analyzed the male individuals discordant for these two SNPs. Among the 88 subjects who have the major allele at rs661426, there were only 2 subjects who have the minor allele at rs2295868, and both have ASD. Among the 190 subjects who have the major allele at rs2295868, there were 95 who have the minor allele at rs661426. Of these, 50 (53.2%) were cases and 45 (46.9%) were controls (p=0.47, OR 1.29). Based on these analyses, rs661426 does not seem to contribute an independent risk of ASD, and rather, the increased risk of ASD is conferred by rs2295868.

Enhancer binding at the *ARHGEF6* locus

We found that the rs2295868 locus was in close proximity (about 1 kb) to an active enhancer region in the intron of the *ARHGEF6* gene, which was marked by the co-localization of H3K4me1, H3K27ac and p300 [21] (Figure 2). Active enhancers are sites for binding of a variety of transcription factors that are important in activating transcription. The existence of variation within the enhancer region of *ARHGEF6* raises the possibility that the transcriptional status of this gene is linked to the development of ASD. However, it remains to be investigated if expression of *ARHGEF6* is reduced in patients. Future experiments to study the gene expression are warranted.

Discussion

In this study we investigated the relationship between polymorphisms in multiple ASD risk genes. We identified one SNP rs2295868 in the *ARHGEF6* gene to be associated with a risk of ASD. The OR for SNP rs2295868 in regard to autism risk is 4.09, which is higher than any other single SNP associated with autism risk [14,22-26]. Arhgef6, also known as αPIX or Cool-2, belongs to a family of GTPases. *ARHGEF6* is expressed in multiple different tissues, but its highest levels are centered in the immune system, the brain, and the intestine [27,28]. In the brain *ARHGEF6* is selectively expressed in the hippocampus CA1 region, which is important for new memory
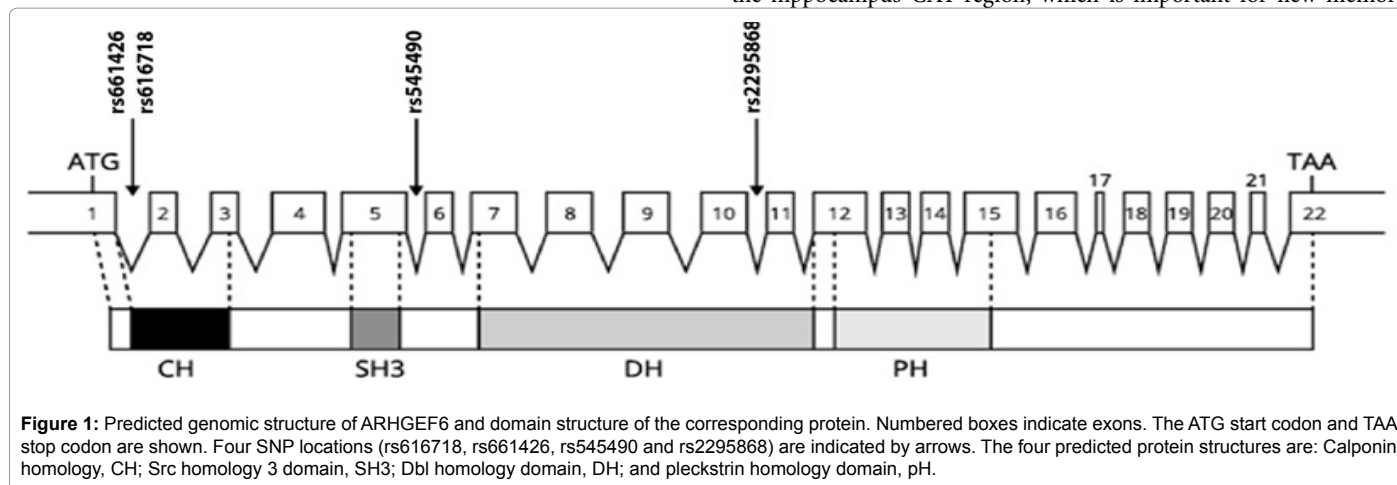

Figure 1: Predicted genomic structure of ARHGEF6 and domain structure of the corresponding protein. Numbered boxes indicate exons. The ATG start codon and TAA stop codon are shown. Four SNP locations (rs616718, rs661426, rs545490 and rs2295868) are indicated by arrows. The four predicted protein structures are: Calponin homology, CH; Src homology 3 domain, SH3; Dbl homology domain, DH; and pleckstrin homology domain, pH.

formation [29]. *ARHGEF6* acts as a molecular switch for the Rho GTPases Rac1 and Cdc42 [30]. Rac1 and Cdc42 are key regulators of the actin cytoskeleton and affect diverse cellular processes, such as adhesion and migration, phagocytosis, cytokinesis, cell polarity, cell growth and survival, and neuronal morphogenesis [31,32]. *ARHGEF6* is also an important promoter for the recycling of epidermal growth factor receptor (EGFR). *ARHGEF6* is part of the Rho GTPase family of proteins, which are known for their role in neurite outgrowth, branching, axon pathfinding, and dendritic spine morphogenesis [33,34]. *ARHGEF6* performs different roles depending on the tissue on which it is being expressed. In the brain *ARHGEF6* is expressed in the hippocampal neuropil. *ARHGEF6* helps to control neurological spine and neurite growth morphologies. Because of its role in neuronal growth, loss of *ARHGEF6* activity results in the decrease in the density of cortical pyramidal neurons [27,30,35]. These mismanaged growth morphologies can lead to deficits in autophagy in microglia and impaired synaptic pruning, which have been linked to behaviors similar to those of ASD [36]. These deficits may contribute to the link between *ARHGEF6* mutations and mental retardation [37]. One other role of *ARHGEF6* induces apoptosis in cells that are experiencing oxidative stress. *ARHGEF6* acts on TP53, HSPA1A, and CFLAR to include apoptosis [38]. One hypothesis for how *ARHGEF6* mutations could increase risk of autism is that patients with these mutations are more chemically sensitive.

Other proteins involved in a signaling pathway with *ARHGEF6* might also play a role in the ASD-risk mechanism. One potential candidate is Fibroblast growth factor 2 (FGF-2) which is a genetic regulator of *ARHGEF6*. Knockout of FGF-2 results in a significant downregulation of mRNA in the brain [27], and has been linked to autism [39,40]. The FGF-2/*ARHGEF6* pathway leads to the activation of Rac1 and Cdc42 [30,41,42]. Decreases in Rac1 activation leads to reduced behavioral flexibility, which is a hallmark autism characteristic [43,44]. Recent studies have investigated the efficacy of fibroblast growth factors in the treatment of autism [45-47].

Studies have indicated that females are protected from ASD. Population screens [48-50] and high-risk sibling [48,51] studies demonstrate that there is a male-biased prevalence for ASD and that some risk factors impact males and females differently. The multiple threshold liability model of ASD, where sex is one factor, seems to indicate the existence of a "female protective effect" (FPE) [52]. The current study supports the FPE hypothesis, because of the much higher OR for autism in males with the rs2295868 allele than in females. However, because it is an X-linked gene, females with the *ARHGEF6* rs2295868 SNP may be carriers for autism risk. In addition to males having a higher risk for autism with the rs2295868 allele, over half of the males with this allele who were not autistic had a dignosis of depression and anxiety disorder.

One of the most common comorbidities of autism is gastrointestinal symptoms (GIS). Reports estimate that the frequency of GIS in the autistic community is approximately 40% [53,54]. Common symptoms are constipation, diarrhea, abdominal pain, and gastroesophageal reflux [55]. A recent study indicates that *ARHGEF6* plays a role in these symptoms [28]. Chang et al. found that *ARHGEF6* plays a role in autoimmune disease in the gut. They performed an X-chromosome wide association study and found that *ARHGEF6* was associated with Crohn's disease and ulcerative colitis. *ARHGEF6* was also found to be highly expressed in T-cells [28]. If *ARHGEF6* does play a role in GIS, it may explain why ASD and GIS are associated.

The mechanisms by which enhancers of *ARHGEF6* might regulate its transcription levels are multifaceted. Some enhancers can directly interact with the gene's promoter through enhancer-promoter looping interactions to activate transcription [56]. Studying whether there is crosstalk between the active enhancer and promoter of *ARHGEF6* will shed light on the potential mechanism of gene activation. In addition, the role of such interactions is not only important for transcription initiation but might also be essential in transcription elongation by releasing RNA polymerase II from promoter-proximal pausing [57]. Moreover, it has been reported that some enhancers are also transcribed into non-coding RNAs, known as enhancer RNAs, which could orchestrate regulation of transcription by stabilizing long-range enhancer-promoter interactions [58]. Examining how the *ARHGEF6* enhancer is able to regulate transcriptional activity will allow us to decipher the molecular etiology of ASD, which might lead to potential therapeutic strategies in ameliorating the disease.
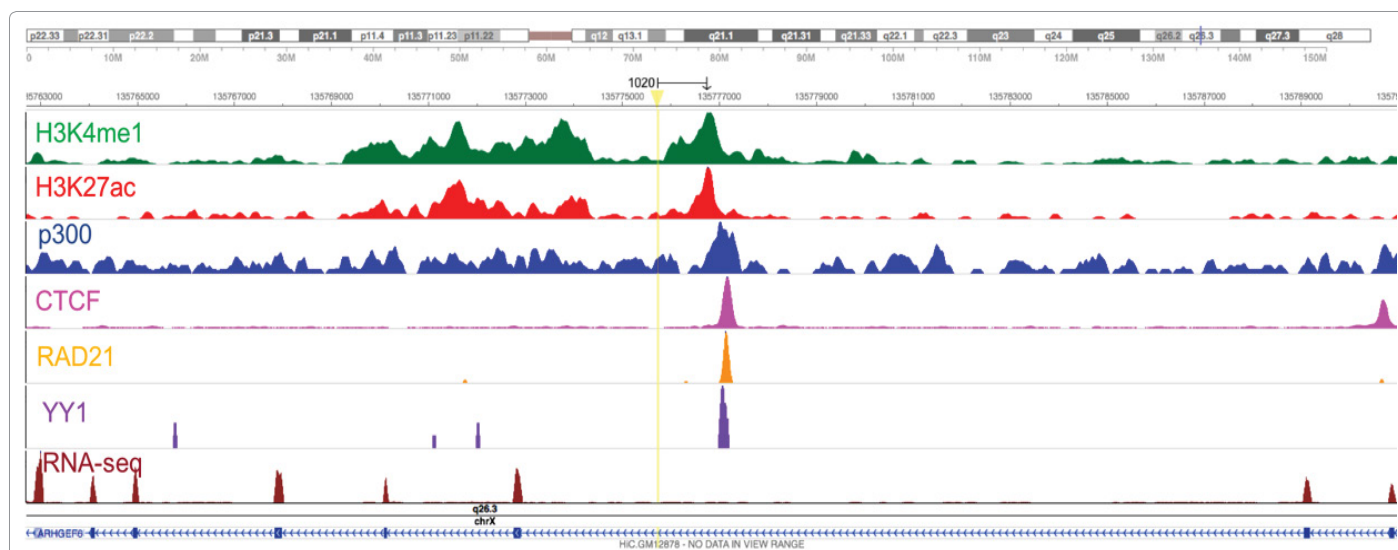

Figure 2: H3K4me1, H3K27ac and p300 are all active enhancer markers. Further from the enhancer region are the binding sites of CTCF, cohesin and YY1. RNA-seq data is to show the exons of the gene.

Gender	SNP	MAF in 1000 genomes	MAF in controls	MAF in cases	p-value	OR	
Males	rs2295868	0.161	0.094	0.299	2.31×10^{-4}	4.09	
	rs661426	0.234	0.519	0.657	0.03	1.77	
	rs616718	0.313	0.311	0.214	0.14	0.60	
	SNP	**MAF in 1000 genomes**	**MAF in controls**	**MAF in cases**	**p-value**	**OR_{het}**	**OR_{hom}**
Females	rs2295868	0.161	0.220	0.214	0.99	1.02	0.84
	rs661426	0.234	0.525	0.595	0.71	1.03	1.64
	rs616718	0.313	0.213	0.167	0.36	0.57	1.15
MAF=Minor Allele Frequency, OR=Odds Ratio							

Table 1: Autism case-control association analysis.

Conclusion

This study showed evidence supporting a role of the *ARHGEF6* gene in the etiology of ASD. The SNP rs2295868 is located near a transcriptional enhancer site, and this polymorphism may cause a decrease in transcription of the *ARHGEF6* mRNA. A decrease of *ARHGEF6* mRNA may lead to multiple pathway changes because of *ARHGEF6*'s multifaceted role. Future studies should be done on other X-linked genes that interact with Rho GTPases such as GFD1, OPHN1, PAK3, and ARHGEF9. Other avenues of future study would be to look at patients with the rs2295868 SNP to see if patients have altered metabolic markers. Looking into markers such as glutathione status, mitochondrial dysfunction, or impaired energy production could produce significant results.

References

1. American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders, 5th edn., Washington, DC, USA.

2. State MW, Levitt P (2011) The conundrums of understanding genetic risks for autism spectrum disorders. Nat Neurosci 14: 1499-1506.

3. Ronald A, Happe F, Bolton P, Butcher LM, Price TS, et al. (2006) Genetic heterogeneity between the three components of the autism spectrum: A twin study. J Am Acad Child Adolesc Psychiatry 45: 691-699.

4. Campbell DB, Buie TM, Winter H, Bauman M, Sutcliffe JS, et al. (2009) Distinct genetic risk based on association of MET in families with co-occurring autism and gastrointestinal conditions. Pediatrics 123: 1018-1024.

5. Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, et al. (1995) Autism as a strongly genetic disorder: Evidence from a British twin study. Psychol Med 25: 63-77.

6. Le-Couteur A, Bailey A, Goode S, Pickles A, Robertson S, et al. (1996) A broader phenotype of autism: The clinical spectrum in twins. J Child Psychol Psychiatry 37: 785-801.

7. Christensen DL, Baio J, Braun VNK, Bilder D, Charles J, et al. (2016) Prevalence and characteristics of autism spectrum disorder among children aged 8 years-autism and developmental disabilities monitoring network, 11 sites, United States, 2012. MMWR Surveill Summ 65: 1-23.

8. Christensen DL, Bilder DA, Zahorodny W, Pettygrove S, Durkin MS (2016) Prevalence and characteristics of autism spectrum disorder among 4-year-old children in the Autism and developmental disabilities monitoring network. J Dev Behav Pediatr 37: 80-82.

9. Raymond FL (2006) X-linked mental retardation: A clinical guide. J Med Genet 43: 193-200.

10. Mitra I, Tsang K, Ladd-Acosta C, Croen LA, Aldinger KA, et al. (2016) Pleiotropic mechanisms indicated for sex differences in Autism. PLoS Genet 12: e1006425.

11. Ning Z, McLellan AS, Ball M, Wynne F, O'Neill C, et al. (2015) Regulation of SPRY3 by X chromosome and PAR2 linked promoters in an autism susceptibility region. Hum Mol Genet 24: 745.

12. Laumonnier F, Cuthbert PC, Grant SG (2007) The role of neuronal complexes in human X-linked brain diseases. Am J Hum Genet 80: 205-220.

13. Skuse DH (2005) X-linked genes and mental functioning. Hum Mol Genet 14: 27-32.

14. Chakrabarti B, Dudbridge F, Kent L, Wheelwright S, Hill-Cawthorne G, et al. (2009) Genes related to sex steroids, neural growth, and social-emotional behavior are associated with autistic traits, empathy, and Asperger syndrome. Autism Res 2: 157-177.

15. Geschwind DH, Levitt P (2007) Autism spectrum disorders: Developmental disconnection syndromes. Curr Opin Neurobiol 17: 103-111.

16. Jiang YH, Yuen RK, Jin X, Wang M, Chen N, et al. (2013) Detection of clinically relevant genetic variants in autism spectrum disorder by whole-genome sequencing. Am J Hum Genet 93: 249-263.

17. Team RC (2013) R foundation for statisical computing, Vienna, Austria.

18. Machiela MJ, Chanock SJ (2015) LD-link: A web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. Bioinformatics 31: 3555-3557.

19. Yuen KC, Xu B, Krantz ID, Gerton JL (2016) NIPBL controls RNA biogenesis to prevent activation of the stress kinase PKR. Cell Rep 14: 93-102.

20. 1000 Genomes Project Consortium (2015) A global reference for human genetic variation. Nature 526: 68-74.

21. Ong CT, Corces VG (2011) Enhancer function: New insights into the regulation of tissue-specific gene expression. Nat Rev Genet 12: 283-293.

22. Yim SV, Kim SK, Park HJ, Jeon HS, Jo BC, et al. (2013) Assessment of the correlation between TIMP4 SNPs and schizophrenia and autism spectrum disorders. Mol Med Rep 7: 489-494.

23. Bowers K, Li Q, Bressler J, Avramopoulos D, Newschaffer C, et al. (2011) Glutathione pathway gene variation and risk of autism spectrum disorders. J Neurodev Disord 3: 132-143.

24. Carayol J, Schellenberg GD, Tores F, Hager J, Ziegler A, et al. (2010) Assessing the impact of a combined analysis of four common low-risk genetic variants on autism risk. Mol Autism 1: 4.

25. Pu D, Shen Y, Wu J (2013) Association between MTHFR gene polymorphisms and the risk of autism spectrum disorders: A meta-analysis. Autism Res 6: 384-392.

26. Romero-Garcia R, Warrier V, Bullmore ET, Baron-Cohen S, Bethlehem RAI (2018) Synaptic and transcriptionally downregulated genes are associated with cortical thickness differences in autism. Mol Psychiatry 2: 1.

27. Baum P, Vogt MA, Gass P, Unsicker K, Von Bohlen und Halbach O (2016) FGF-2 deficiency causes dysregulation of Arhgef6 and downstream targets in the cerebral cortex accompanied by altered neurite outgrowth and dendritic spine morphology. Int J Dev Neurosci 50: 55-64.

28. Chang D, Gao F, Slavney A, Ma L, Waldman YY, et al. (2014) Accounting for eXentricities: Analysis of the X chromosome in GWAS reveals X-linked genes implicated in autoimmune diseases. PLoS One 9: 113684.

29. Meyer MA (2014) Highly expressed genes within hippocampal sector CA1: Implications for the physiology of memory. Neurol Int 6: 5388.

30. Ramakers GJ, Wolfer D, Rosenberger G, Kuchenbecker K, Kreienkamp HJ, et al. (2012) Dysregulation of Rho GTPases in the alphaPix/Arhgef6 mouse model of X-linked intellectual disability is paralleled by impaired structural and synaptic plasticity and cognitive deficits. Hum Mol Genet 21: 268-286.

31. Etienne-Manneville S, Hall A (2002) Rho GTPases in cell biology. Nature 420: 629-635.

32. Heasman SJ, Ridley AJ (2008) Mammalian Rho GTPases: New insights into their functions from *in vivo* studies. Nat Rev Mol Cell Biol 9: 690-701.

33. Govek EE, Newey SE, Van Aelst L (2005) The role of the Rho GTPases in neuronal development. Genes Dev 19: 1-49.

34. Luo L (2002) Actin cytoskeleton regulation in neuronal morphogenesis and structural plasticity. Annu Rev Cell Dev Biol 18: 601-635.

35. Node-Langlois R, Muller D, Boda B (2006) Sequential implication of the mental retardation proteins ARHGEF6 and PAK3 in spine morphogenesis. J Cell Sci 119: 4986-4993.

36. Kim HJ, Cho MH, Shim WH, Kim JK, Jeon EY, et al. (2017) Deficient autophagy in microglia impairs synaptic pruning and causes social behavioral defects. Mol Psychiatry 22: 1576-1584.

37. Kutsche K, Yntema H, Brandt A, Jantke I, Nothwang HG, et al. (2000) Mutations in ARHGEF6, encoding a guanine nucleotide exchange factor for Rho GTPases, in patients with X-linked mental retardation. Nat Genet 26: 247-250.

38. Maiti AK (2010) Gene network analysis of oxidative stress-mediated drug sensitivity in resistant ovarian carcinoma cells. Pharmacogenomics J 10: 94-104.

39. Meng L, Lu L, Murphy KM, Yuede CM, Cheverud JM, et al. (2011) Neuroanatomic and behavioral traits for autistic disorders in age specific restricted index selection mice. Neuroscience 189: 215-222.

40. Esnafoglu E, Ayyildiz SN (2017) Decreased levels of serum fibroblast growth factor-2 in children with autism spectrum disorder. Psychiatry Res 257: 79-83.

41. Baird D, Feng Q, Cerione RA (2005) The Cool-2/alpha-Pix protein mediates a Cdc42-Rac signaling cascade. Curr Biol 15: 1-10.

42. Feng Q, Albeck JG, Cerione RA, Yang W (2002) Regulation of the Cool/Pix proteins: Key binding partners of the Cdc42/Rac targets, the p21-activated kianses. J Biol Chem 277: 5644-5650.

43. Dong T, He J, Wang S, Wang L, Cheng Y, et al. (2016) Inability to activate Rac1-dependent forgetting contributes to behavioral inflexibility in mutants of multiple autism-risk genes. Proc Natl Acad Sci USA 113: 7644-7649.

44. Zeidan-Chulia F, Rybarczyk-Filho JL, Salmina AB, De Oliveira BH, Noda M, et al. (2013) Exploring the multifactorial nature of autism through computational systems biology: Calcium and the Rho GTPase RAC1 under the spotlight. Neuromolecular Med 15: 364-383.

45. Terauchi A, Gavin E, Wilson J, Umemori H (2017) Selective inactivation of fibroblast growth factor 22 (FGF22) in CA3 pyramidal neurons impairs local synaptogenesis and affective behavior without affecting dentate neurogenesis. Front Synaptic Neurosci 19: 9-17.

46. Li Q, Chen CF, Wang DY, Lu YT, Huan YL, et al. (2015) Transplantation of umbilical cord blood mononuclear cells increases levels of nerve growth factor in the cerebrospinal fluid of patients with autism. Genet Mol Res 14: 8725-8732.

47. Vaccarino FM, Grigorenko EL, Smith KM, Stevens HE (2009) Regulation of cerebral cortical size and neuron number by fibroblast growth factors: Implications for autism. J Autism Dev Disord 39: 511-520.

48. Idring S, Magnusson C, Lundberg M, Ek M, Rai D, et al. (2014) Parental age and the risk of autism spectrum disorders: Findings from a Swedish population-based cohort. Int J Epidemiol 43: 107-115.

49. Kawamura Y, Takahashi O, Ishii T (2008) Re-evaluating the incidence of pervasive developmental disorders: Impact of elevated rates of detection through implementation of an integrated system of screening in Toyota, Japan. Psychiatry Clin Neurosci 62: 152-159.

50. Kim YS, Leventhal BL, Koh YJ, Fombonne E, Laska E, et al. (2011) Prevalence of autism spectrum disorders in a total population sample. Am J Psychiatry 16: 904-912.

51. Ozonoff S, Young GS, Carter A, Messinger D, Yirmiya N, et al. (2011) Recurrence risk for autism spectrum disorders: A baby siblings research consortium study. Pediatrics 128: 488-495.

52. Werling DM, Geschwind DH (2013) Sex differences in autism spectrum disorders. Curr Opin Neurol 26: 146-153.

53. Valicenti-McDermott M, McVicar K, Rapin I, Wershil BK, Cohen H, et al. (2006) Frequency of gastrointestinal symptoms in children with autistic spectrum disorders and association with family history of autoimmune disease. J Dev Behav Pediatr 27: 128-136.

54. Wang LW, Tancredi DJ, Thomas DW (2011) The prevalence of gastrointestinal problems in children across the United States with autism spectrum disorders from families with multiple affected members. J Dev Behav Pediatr 32: 351-360.

55. Horvath K, Perman JA (2002) Autism and gastrointestinal symptoms. Curr Gastroenterol Rep 4: 251-258.

56. Kagey MH, Newman JJ, Bilodeau S, Zhan Y, Orlando DA, et al. (2010) Mediator and cohesin connect gene expression and chromatin architecture. Nature 467: 430-435.

57. Zippo A, Serafini R, Rocchigiani M, Pennacchini S, Krepelova A, et al. (2009) Histone crosstalk between H3S10ph and H4K16ac generates a histone code that mediates transcription elongation. Cell 138: 1122-1136.

58. Orom UA, Derrien T, Beringer M, Gumireddy K, Gardini A, et al. (2010) Long noncoding RNAs with enhancer-like function in human cells. Cell 143: 46-58.