

Involvement of Voltage-Gated Ca^{2+} Channels in Autism Spectrum Disorders

Angela L. Wang;¹ Feng Liu, MD, PhD;² Gang Wang, MD, PhD^{3*}

¹ Premed student, Washington University in St. Louis, St. Louis, MO

² Department of Child & Adolescent Psychiatry, New York University-Bellevue Hospital Center, New York, NY

³ Brain and Mind Research Institute, Weill Cornell Medical College, New York, NY

Substantial evidence suggests that mutated voltage-gated L-type Ca^{2+} channel subunit $\text{Ca}_v1.2$ (*CACNA1C*) is involved in pathophysiology of autism spectrum disorder (ASD), as exemplified by recent findings that mutated *CACNA1C* and other L-type Ca^{2+} channels play an important role in pathophysiology of Timothy Syndrome and Fragile X Syndrome, genetic syndromes of ASD. This review will focus on recent advances in our understanding of the genetic mutation of the $\text{Ca}_v1.2$ L-type channels in ASD, and highlight the potential roles of $\text{Ca}_v1.2$ channels in the pathogenesis of ASD.

[N A J Med Sci. 2014;7(3):135-138. DOI: 10.7156/najms.2014.0703135]

Key Words: autism spectrum disorders, Ca^{2+} channels, Timothy syndrome, Fragile X syndrome

AUTISM SPECTRUM DISORDERS

Autism was once known as childhood schizophrenia, and two disorders were not clearly differentiated until the 1970s. Autism is a group of developmental brain disorders, collectively called autism spectrum disorders (ASD). The term "spectrum" refers to the wide range of symptoms, and levels of impairment or disability that children with ASD could have. Some children are mildly impaired by their symptoms, but others are severely disabled. One of the most important changes in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) is autism spectrum disorder, a single disorder name that may improve the diagnosis of ASD without limiting the sensitivity of the criteria, or substantially changing the number of children be diagnosed.^{10-12,15,17,27}

People with ASD tend to have communication deficits, such as responding inappropriately in conversations, misreading nonverbal interactions, or having difficulty building friendships appropriate to their age. In addition, people with ASD may be overly dependent on routines, highly sensitive to changes in their environment, or intensely focused on inappropriate items. Because of the unique mixture of symptoms shown in each child, severity level can sometimes be difficult to determine.¹⁷ Individuals with ASD may show symptoms from early childhood, yet those symptoms may not appear until later. Since an accurate diagnosis and early identification of ASD can provide the basis for building an appropriate and effective educational and treatment program, and providing a better prognosis, it is therefore imperative to take advantage of recently discovered genetic mutations and

other laboratory biomarkers for ASD, with any early clinical symptoms of this disease, to make diagnosis of ASD as early as possible. This review will focus on the ASD-linked Ca^{2+} channel mutations and dysfunctions identified recently in animal model as signs of ASD, such as repetitive and perseverative behavior, altered social behavior, and enhanced contextual memory following fear conditioning.¹

THE LINK BETWEEN GENETIC MUTATION OF VOLTAGE-GATED L-TYPE Ca^{2+} CHANNEL $\text{Ca}_v1.2$ (*CACNA1C*) AND ASD OR OTHER PSYCHIATRIC DISORDERS

The brain mainly expresses two high threshold voltage-activated L-type Ca^{2+} channels (VALTCC), $\text{Ca}_v1.2$ and $\text{Ca}_v1.3$.³ $\text{Ca}_v1.2$ is involved in regulation of blood pressure and serves as a key signal for the excitability, long-term plasticity, memory and gene expression.³ Considerable evidence implicates dysfunction of VALTCC in the pathophysiology of ASD, bipolar disorders, major depression and schizophrenia.^{3,6,7,19,21,25,26}

Over the last ten years, genome-wide association studies (GWAS) have identified a strong association signal for bipolar disorder with *CACNA1C*, the gene encoding the $\text{Ca}_v1.2$ channel, suggesting that bipolar disorder is, at least in part, an ion channelopathy. The maximal signal of a single nucleotide polymorphism (SNP) rs1006737 within intron 3 of the *CACNA1C* in chromosome 12p13.3 has been identified in bipolar disorder patients.⁹ The risk allele SNP rs1006737 predicted increased expression of *CACNA1C* mRNA in human brain.⁹ Using functional magnetic resonance imaging to measure brain activation in neural circuits, increased activities in the prefrontal cortex during executive cognition, in the hippocampus during emotional processing and in the amygdala in response to reward were observed in the healthy

Received: 06/12/2014; Revised: 06/29/2014; Accepted: 07/13/2014

*Corresponding Authors: Brain and Mind Research Institute, Weill Cornell Medical College, New York, NY.

(Email: gaw2001@med.cornell.edu)

carriers of the risk allele SNP rs1006737. However, decreased activities in the bilateral hippocampus during episodic memory recall, in the subgenual anterior cingulate cortex, in the right inferior parietal lobule during orienting and in the medial frontal gyrus during executive control of attention were described.^{8,14} Furthermore, the association of bipolar disorder drug target genes with *CACNA1C* is also supported by other report that the mRNA of *CACNA1C* in the brain was downregulated in mouse brain in response to lithium treatment for bipolar disorder.⁹ Dihydropyridine antagonists of *CACNA1C* exerted antidepressant-like effects by reducing the immobility in mouse behavioral despair models.³

More interestingly, the largest GWAS yet has analyzed SNP for the five psychiatric disorders among 33,332 cases and 27,888 controls, all of European ancestry.⁶ Four independent regions contained SNP that were significantly related to essential (or non-syndromic) ASD, schizophrenia, major depressive disorder, bipolar disorder and attention deficit-hyperactivity disorder. Three loci were related to all five disorders, and one to only bipolar disorder and schizophrenia. Two loci are related to VALTCC and one is *CACNA1C* but with a different SNP (rs1024582).⁶

In addition, the gene coding for the L-type Ca^{2+} channel subunits $\text{Ca}_v\beta 2$ (*CACNB2*) were recently identified as risk loci for ASD.^{4,6} Three rare missense mutations of *CACNB2* (G167S, S197F, and F240L) found in ASD-affected families may be of importance, since these missense mutations modulate whole-cell Ba^{2+} currents via Ca^{2+} channels expressed in HEK-293 cells. Two mutations displayed significantly decelerated time-dependent inactivation as well as increased sensitivity of voltage-dependent inactivation. In contrast, the third mutation (F240L) showed significantly accelerated time-dependent inactivation.⁴ These results for the first time demonstrates the functional changes in Ca^{2+} influx associated with these three rare *CACNB2* mutations in ASD patients, supporting the notion that Ca^{2+} channel dysfunction contributes to ASD. Taken together, alterations in Ca^{2+} channel signaling may represent a fundamental mechanism contributing to the vulnerability to ASD.

MUTATIONS OF L-TYPE Ca^{2+} CHANNELS $\text{Ca}_v1.2$ INDUCES GATING AND BEHAVIORAL CHANGES IN ASD MODELS

Recently, accumulating evidence also shows that functional mutations in the *CACNA1C* gene contribute to ASD induced by gene-mutations (or syndromic ASD).^{2,3,6,23} One typical example is Timothy Syndrome (TS), a rare human autosomal-dominant ASD, which presents with long Q-T syndrome, congenital heart disease, and ASD symptoms (80% patients who survive long enough for psychiatric evaluation). TS is associated with a sporadic glycine-to-arginine mutation of G406R in the cytoplasmic end of the exon 8A-coded transmembrane segment IS6 in the exon 8A splice variant of *CACNA1C*.^{1,2,23,24} Functionally, this mutation greatly delays voltage-dependent inactivation (closing) of the *CACNA1C* Ca^{2+} channel,² and thus may potentially cause a pathogenic calcium overload in cardiac

and neuronal cells. An analogous mutation in alternative exon 8 (mutually exclusive with exon 8A), G402S, and a corresponding reduction in voltage-dependent inactivation were observed in $\text{Ca}_v1.2$ of a Timothy Syndrome variant.²⁴

The milder TS1 and the more severe TS2 variants arise from the same missense mutations, but TS2 variant is more severe due to a glycine-to-arginine mutation occurring in the more highly (~80%) expressed alternative exon 8 in alternatively spliced exons that cause the same G406R replacement in the $\text{Ca}_v1.2$ channel.²² A neomycin cassette (TS2-neo), inverted to keep the TS mutant $\text{Ca}_v1.2$ channel less expressed to avoid fatality, is able to make TS2-like mouse survive through adulthood, allowing a thorough behavioral phenotyping.¹ Using this unique viable ASD model, it has been observed that with normal general health, activity and anxiety level, TS2-neo mice showed markedly restricted, repetitive, and perseverative behavior, altered social behavior, altered ultrasonic vocalization, and enhanced contextual memory following fear conditioning,¹ suggesting that low expression of TS mutant $\text{Ca}_v1.2$ channels, while avoiding fatality, is sufficient to cause multiple, distinct behavioral abnormalities that are in agreement with the core aspects of ASD.

However, in Fragile X Syndrome (FXS), another case of gene-linked syndromic ASD, there is a decreased expression of L-type Ca^{2+} channels in central neurons.^{5,18} FXS, the most widespread single-gene cause of autism with mental retardation, is associated with the expansion of the CGG trinucleotide repeat in the Fragile X mental retardation 1 (*FMR1*) gene on the X chromosome, which results in a failure to express the fragile X mental retardation protein (FMRP) that is required for normal neural development.

Western blot assays in wild type and FMRP1 knockout mice demonstrated that the levels of the $\text{Ca}_v1.2$ subunit were reduced in FXS human brains. Specifically, expression of $\text{Ca}_v1.2$ subunit was reduced in all tested brain regions and ages as well as in cortical synaptosomes of 2 week old FXS mice.⁵ Furthermore, it is reported that excitatory postsynaptic currents (EPSC) and long-term potentiation (LTP) in the animal model of FXS were also attenuated.¹⁸ In parallel, postsynaptic calcium signaling in FMR1 knockout (KO) mice is compromised in two ways: first, dendrites and spines of *FMR1*-KO neurons in pyramidal neurons located in L2/3 of the middle prefrontal cortex (mPFC) more often fail to show Ca^{2+} transients in response to single action potentials or bursts of action potentials than WT neurons. Second, in contrast to spines of WT neurons, voltage-gated L-type Ca^{2+} channels do not contribute significantly to calcium transients in dendritic spines of *FMR1*-KO neurons.¹⁸ This is in line with their finding that the contribution of functional L-type Ca^{2+} channels to dendritic spine calcium dynamics is strongly reduced,¹⁸ and the finding by Chen et al that $\text{Ca}_v1.2$ subunit was reduced in all tested brain regions and ages as well as in cortical synaptosomes of fragile X mice.⁵ Taken together, these results suggest that the attenuated expression of postsynaptic L-type Ca^{2+} channels in FXS brains may be

responsible for post-synaptic calcium signaling in *FMR1*-KO models.

POSSIBLE ROLES OF T-TYPE VOLTAGE-GATED Ca^{2+} CHANNELS IN ASD

Other voltage-gated Ca^{2+} channels such as T-type Ca^{2+} channels may also be involved in non-syndromic forms of ASD. T-type Ca^{2+} channels activate with relatively small depolarization of the neuron membrane triggering low threshold spikes that contribute to rebound burst firing and oscillatory behavior in central neurons, therefore playing a crucial role in determining neuronal excitability and are involved in sensory processing and pathophysiology of epilepsy.¹³ Substitutions in highly conserved residues of T-type $\text{Ca}_v3.2$ (*CACNA1H*) genes in six of 461 patients with ASD were reported.²⁴ These mutations altered the functional properties of the T-type Ca^{2+} channel currents.²⁴ On the other hand, neuroanatomical studies of autistic patients have found $\text{Ca}_v3.2$, a T-type Ca^{2+} channel encoded by the *CACNA1H* gene is abundantly expressed in the hippocampus and amygdala and in the cerebellum and cerebral cortex.²⁰ In a separate study using the samples from the Autism Genetics Research Exchange collection, the rs198538 SNP located in the *CACNA1G* ($\text{Ca}_v3.1$) and rs5750860 SNP located in the *CACNA1I* ($\text{Ca}_v3.3$) gene identified in this study is located within a portion of the genome that can be pulled down by Egr1 chromatin immunoprecipitation.¹⁶

CONCLUDING REMARKS

It is widely accepted that the interplay of genetic, developmental and environmental factors contributes to the pathogenesis of ASD. *CACNA1C* gene encoding $\text{Ca}_v1.2$ VALTCC is a strong candidate as one of susceptibility genes for ASD. This is of particular interests, because replication of this susceptibility gene is not only obvious in several publications, but also shows future treatments to affect voltage-activated L-type calcium channel functioning might have effects across a range of disorders including bipolar disorder and ASD.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

GLOSSARY

Anxiety disorder: a group of disabling disorders characterized by excessive, irrational anxiety or fears that have abnormally increased intensity and duration, and are egodystonic.

Bipolar disorder: characterized by functioning-impairing intermittent episodes of mania or hypomania, usually interlaced with depressive episodes.

Major depressive disorder: a common mental disorder characterized by depressed mood accompanied by anhedonia, feeling of guilty or hopelessness, change of appetite and weight; low energy, poor concentration, psychomotor retardation or agitation, and suicidal ideation.

Genome-wide association study (GWAS): is an examination in genetic epidemiology of all or most of the genome of different individuals of a particular species to see how much the genes vary from individual to individual. Different variations are then associated with different traits or diseases.

Single nucleotide polymorphism (SNP): is a DNA sequence variation occurring when a single nucleotide -A, T, C, or G - in the genome differs between members of a biological species or paired chromosomes in an individual.

REFERENCES

1. Bader PL, Faizi M, Kim LH, et al. Mouse model of Timothy syndrome recapitulates triad of autistic traits. *Proc Natl Acad Sci USA*. 2011; 108(37):15432-15437.
2. Barrett CF, Tsien RW. The Timothy syndrome mutation differentially affects voltage- and calcium-dependent inactivation of $\text{Ca}_v1.2$ L-type calcium channels. *Proc Natl Acad Sci USA*. 2008;105(6):2157-2162.
3. Bhat S, Dao DT, Terrillion CE, et al. *CACNA1C* ($\text{Ca}_v1.2$) in the pathophysiology of psychiatric disease. *Prog Neurobiol*. 2012; 99(1):1-14.
4. Breitenkamp AF, Matthes J, Nass RD, et al. Rare Mutations of *CACNB2* Found in Autism Spectrum Disease-Affected Families Alter Calcium Channel Function. *PLoS One*. 2014; 9(4): e95579.
5. Chen L, Yun SW, Seto J, Liu W, Toth M. The fragile X mental retardation protein binds and regulates a novel class of mRNAs containing U rich target sequences. *Neuroscience*. 2003;120(4):1005-1017.
6. Consortium C-DGPG. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *Lancet*. 2013; 381(9875):1371-1379.
7. Ferreira MA, O'Donovan MC, Meng YA, et al. Collaborative genome-wide association analysis supports a role for *ANKK3* and *CACNA1C* in bipolar disorder. *Nat Genet*. 2008;40(9):1056-1058.
8. Franke B, Vasquez AA, Veltman JA, Brunner HG, Rijkema M, Fernandez G. Genetic variation in *CACNA1C*, a gene associated with bipolar disorder, influences brainstem rather than gray matter volume in healthy individuals. *Biol Psychiatry*. 2010;68(6):586-588.
9. Gershon ES, Grennan K, Busnello J, et al. A rare mutation of *CACNA1C* in a patient with bipolar disorder, and decreased gene expression associated with a bipolar-associated common SNP of *CACNA1C* in brain. *Mol Psychiatry*. 2014;19(8):890-894.
10. Guthrie W, Swineford LB, Wetherby AM, Lord C. Comparison of DSM-IV and DSM-5 factor structure models for toddlers with autism spectrum disorder. *J Am Acad Child Adolesc Psychiatry*. 2013; 52(8):797-805.
11. Harrington JW, Allen K. The clinician's guide to autism. *Pediatr Rev*. 2014;35(2):62-78.
12. Huerta M, Bishop SL, Duncan A, Hus V, Lord C. Application of DSM-5 criteria for autism spectrum disorder to three samples of children with DSM-IV diagnoses of pervasive developmental disorders. *Am J Psychiatry*. 2012;169(10):1056-1064.
13. Joksovic PM, Nelson MT, Jevtovic-Todorovic V, et al. $\text{Ca}_v3.2$ is the major molecular substrate for redox regulation of T-type Ca^{2+} channels in the rat and mouse thalamus. *J Physiol*. 2006;574(Pt 2):415-430.
14. Kempton MJ, Ruberto G, Vassos E, et al. Effects of the *CACNA1C* risk allele for bipolar disorder on cerebral gray matter volume in healthy individuals. *Am J Psychiatry*. 2009; 166(12):1413-1414.
15. King BH, Navot N, Bernier R, Webb SJ. Update on diagnostic classification in autism. *Curr Opin Psychiatry*. 2014;27(2):105-109.
16. Lu AT, Dai X, Martinez-Agosto JA, Cantor RM. Support for calcium channel gene defects in autism spectrum disorders. *Mol Autism*. 2012;3(1):18.
17. Maenner MJ, Rice CE, Ameson CL, et al. Potential impact of DSM-5 criteria on autism spectrum disorder prevalence estimates. *JAMA Psychiatry*. 2014;71(3):292-300.
18. Meredith RM, Holmgren CD, Weidum M, Burnashev N, Mansvelder HD. Increased threshold for spike-timing-dependent plasticity is caused by unreliable calcium signaling in mice lacking fragile X gene *FMR1*. *Neuron*. 2007;54(4):627-638.
19. Nyegaard M, Demontis D, Foldager L, et al. *CACNA1C* (rs1006737) is associated with schizophrenia. *Mol Psychiatry*. 2010;15(2):119-121.
20. Sadamatsu M, Kanai H, Xu X, Liu Y, Kato N. Review of animal models for autism: implication of thyroid hormone. *Congenit Anom (Kyoto)*. 2006;46(1):1-9.
21. Sklar P, Smoller JW, Fan J, et al. Whole-genome association study of bipolar disorder. *Mol Psychiatry*. 2008;13(6):558-569.
22. Splawski I, Timothy KW, Decher N, et al. Severe arrhythmia disorder caused by cardiac L-type calcium channel mutations. *Proc Natl Acad Sci USA*. 2005;102(23):8089-8096.
23. Splawski I, Timothy KW, Sharpe LM, et al. $\text{Ca}_v1.2$ calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. *Cell*. 2004;119(1):19-31.

24. Splawski I, Yoo DS, Stotz SC, Cherry A, Clapham DE, Keating MT. CACNA1H mutations in autism spectrum disorders. *J Biol Chem*. 2006;281(31):22085-22091.
25. Wessa M, Linke J, Witt SH, et al. The CACNA1C risk variant for bipolar disorder influences limbic activity. *Mol Psychiatry*. 2010;15(12):1126-1127.
26. Wray NR, Pergadia ML, Blackwood DH, et al. Genome-wide association study of major depressive disorder: new results, meta-analysis, and lessons learned. *Mol Psychiatry*. 2010;17(1):36-48.
27. Young RL, Rodi ML. Redefining autism spectrum disorder using DSM-5: the implications of the proposed DSM-5 criteria for autism spectrum disorders. *J Autism Dev Disord*. 2013;44(4):758-765.