SHORT REPORT

Evidence for Interaction Between Markers in GABA(A) Receptor Subunit Genes in an Argentinean Autism Spectrum Disorder Population

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Autism spectrum disorders (ASD) can be conceptualized as a genetic dysfunction that disrupts development and function of brain circuits mediating social cognition and language. At least some forms of ASD may be associated with high level of excitation in neural circuits, and gamma-aminobutyric acid (GABA) has been implicated in its etiology.

Single-nucleotide polymorphisms (SNP) located within the GABA receptor (GABAR) subunit genes GABRA1, GABRG2, GABRB3, and GABRD were screened. A hundred and thirty-six Argentinean ASD patients and 150 controls were studied, and the contribution of the SNPs in the etiology of ASD was evaluated independently and/or through gene–gene interaction using multifactor dimensionality reduction (MDR) method.

From the 18 SNP studied, 11 were not present in our Argentinean population (patients and controls) and 1 SNP had minor allele frequency < 0.1%. For the remaining six SNPs, none provided statistical significant association with ASD when considering allelic or genotypic frequencies. Non-significant association with ASD was found for the haplotype analysis. MDR identified evidence for synergy between markers in GABRB3 (chromosome 15) and GABRD (chromosome 1), suggesting potential gene–gene interaction across chromosomes associated with increased risk for autism (testing balanced accuracy: 0.6081 and cross-validation consistency: 10/10, $P < 0.001$).

Considering our Argentinean ASD sample, it can be inferred that GABRB3 would be involved in the etiology of autism through interaction with GABRD. These results support the hypothesis that GABAR subunit genes are involved in autism, most likely via complex gene–gene interactions. ***Autism Res*** *2013, ••: ••–••.* © 2013 International Society for Autism Research, Wiley Periodicals, Inc.

Keywords: autism spectrum disorder (ASD); GABA(A) receptor subunits polymorphisms; multifactor dimensionality reduction method; Argentinean population

Introduction

Autism spectrum disorder (ASD) (MIM209850) is defined by clinical impairment in language, social interaction, and behavioral flexibility, all prior to 3 years of age. It can be conceptualized as a genetic dysfunction that disrupts development and function of brain circuits mediating social cognition and language [Abrahams & Geschwind, 2010]. Gene–gene interactions are hypothesized to play an important role in its etiology [Ashley-Koch et al., 2006].

Some forms of ASD may be associated with a cortical-disconnectivity model [Gepner & Féron, 2009], which proposes that ASD may be caused by increases/decreases in functional connectivity of relevant pathways [Connolly, Glessner, & Hakonarson, 2013]. More excitable (weakly inhibited) cortex would lead to broad-ranging abnormalities in perception, memory and cognition, motor control, and would be susceptible to epilepsy [Rubenstein & Merzenich, 2003]. Disconnection between different cerebral modulus, as result of congenital disturbance of cerebral development, could lead to altered information processing [for a comprehensive review, see Geschwind & Levitt, 2007]. The neocortical architecture is organized in minicolumns of functionally related glutamatergic and GABAergic neurons processing together thalamic input. GABAergic neurons participate in controlling functional integrity and segregation in minicolumns providing lateral inhibition of activity coming from bordering ones [Casanova, Buxhoeveden, & Gomez, 2003]. Low levels of gamma-aminobutyric acid (GABA) may reduce the threshold for development of epilepsy, as they cause a deficit in inhibitory neurotransmission and, consequently, hyperexcitability of neurons [Fatemi et al., 2002]. There is a long-established history of comorbidity between epilepsy and ASD, and up to 58.5%

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of individuals with ASD have had seizures at some point in their lives [Parmeggiani et al., 2010].

It has been suggested that autistic disorder involves multiple variants in multiple unlinked loci that interact to cause autism phenotype [Ma et al., 2005]. Genome-wide association studies have so far detected no strong contribution of common alleles [State, 2010]. However, in association studies, transmitted rare copy number variants, such as 15q11–q13 duplication, have been found to contribute [Levy et al., 2011]. This chromosome region is of interest as it harbors three GABA receptor (GABAR) subunit genes: GABRB3, GABRA5, and GABRG3 [Cook et al., 1998]. Polymorphisms in GABAR subunits have been associated with epilepsy [Kumari et al., 2011], and GABAergic abnormalities have been involved in ASD [for a comprehensive review, see Rossignol, 2011].

Objectives

The aim of the present work was to study polymorphisms in GABAR subunit genes in a sample of ASD patients from Argentina and to evaluate their contribution to the etiology of ASD independently and/or through complex interactions between genes.

Methods

Participants

A hundred and thirty-six ASD patients (116 males and 20 females) who received a clinical diagnosis of autism by DSM-IV-TR (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision) criteria (75 participants were diagnosed with autism, 32 with Asperger syndrome, and 29 with pervasive developmental disorder not otherwise specified), and 150 healthy controls (128 males and 22 females) from the DNA BioBank, Hospital Italiano de Buenos Aires, were included in the study. Informed consent (approved by an Independent Ethical Committee) was signed by all participants or their parents/legal representative.

Subjects were excluded if they had evidence of developmental disorders with known phenotypic overlap with autism (e.g. Prader–Willi syndrome, Angelman syndrome, tuberous sclerosis complex, Rett syndrome, and fragile X syndrome), neurologic disorders, or severe sensory or motor disorders.

Procedure

Buccal swabs were obtained from all participants, and DNA was extracted in accordance with the manufacturer's instructions using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA).

Eighteen single-nucleotide polymorphisms (SNP) located within the GABAR subunit genes across chromosomes were analyzed. The selected GABAR subunit genes were: GABRA1 (one SNP typed) on chromosome 5, GABRB3 (five SNPs) on chromosome 15, GABRD (nine SNPs) on chromosome 1, and GABRG2 (three SNPs) on chromosome 5.

Polymerase chain reaction primers were designed using the reference sequence for each amplicon from the NCBI database and the online tool Primer-Blast (http://www.ncbi.nlm.nih.gov) (primers sequences available upon request). After amplification, SNPs were genotyped using DNA capillary sequencing (Macrogen Inc., Seoul, Korea).

Statistical Analysis

Genetic association to test whether single-locus allele frequencies, genotype frequencies, and multilocus haplotype frequencies show differences between cases and controls, as well as Hardy–Weinberg equilibrium (HWE) test for each SNP and pair-wise linkage disequilibrium (LD) estimations were performed using SNPStats available from http://bioinfo.iconcologia.net/SNPstats and UNPHASED version 3.1.7 available from https://sites.google.com/site/fdudbridge/software. Testing for genetic interactions in association with autism was performed using the multifactor dimensionality reduction (MDR) method [Hahn, Ritchie, & Moore, 2003], version beta 3.0.2, available from http://www.epistasis.org/mdr%20.html. For more information regarding MDR method, please refer to the Supplementary Material: MDR Method.

Results

None of the 18 SNPs examined deviated from HWE expectations in affected or unaffected individuals in our sample ($P > 0.05$).

In 11 out of the 18 studied SNP, the risk allele was not observed in ASD patients nor controls from our Argentinean sample (GABRA1: rs121434579; GABRB3: rs57294806; GABRD: rs121434580, rs6688232, rs3795278, rs3795279, rs139300921, rs34122464, rs75981360; and GABRG2: rs121909672 rs17855004) and were not included in all posterior analysis. Rs41307846 (GABRD) was also excluded from the analysis as the minor allele frequency was < 0.1% in our sample.

For the remaining 6 out of 18 SNP studied (GABRB3: rs3212337, rs3212338, rs4906902, rs20317; GABRD: rs140480490; and GABRG2 rs211037), the four polymorphic SNPs studied in GABRB3 were in LD ($P < 0.05$), and there was no linkage between markers across the genes studied ($P > 0.05$). None of these six markers provided a statistical significant association with ASD when considering allelic or genotypic frequencies at each marker independently ($P > 0.05$) (Table S1 and S2).

Table 1. MDR Results: Models Detected and Their Performance

No. of markers considered	Best candidate model	CVC[a]	TBA[b]	*P*-value*
1	GABRB3	10/10	0.5712	0.150
2	**GABRB3, GABRD**	**10/10**	**0.6081**	**< 0.001**
3	GABRB3, GABRD, GABRG2	10/10	0.5885	0.054

[a]CVC (cross-validation consistency): measure of how many times out of ten divisions of the data, MDR found the same best model.
[b]TBA (testing balanced accuracy): measure of the predictive performance of the model, the one with the highest TBA represents the best overall model.
**P*-value based on 1000 permutations: shows the statistical significance of the model. The bold font indicate the overall best MDR model.

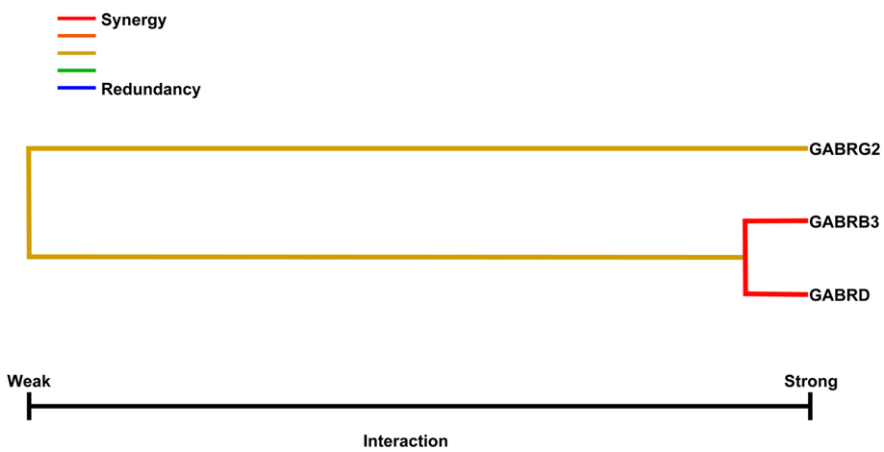

Figure 1. Interaction dendrogram for the markers tested in the multifactor dimensionality reduction (MDR) analysis to evaluate association with autism spectrum disorders (ASD). Interactions between the three markers clearly show a strong synergistic effect (epistasis) between markers in GABRB3 and GABRD, which are the two markers that comprise the overall best MDR model.

Multilocus haplotype analysis showed no significant association with ASD (data not shown). Differences between genders were not found in any analysis.

The results of MDR analysis for each factor considered are presented in Table 1, where it is summarized the average cross-validation consistency (CVC) and average testing balance accuracy (TBA) obtained, and the empirical *P*-value derived from permutation testing. It can be seen that the model that maximized both, TBA and CVC, was the two-factor model that included the genetic variants of GABRB3 and GABRD. This model has a maximum TBA of 0.6081 ($P < 0.001$) indicating the ability to properly classify 60% of the individuals included in the analysis, and a CVC of ten out of ten which denotes that this particular marker combination was chosen by MDR across the ten runs. The interaction dendrogram (Fig. 1) allows a better interpretation of the genetic effects identified by MDR. The dendrogram displays a synergistic effect between the markers of these two genes suggesting a potential gene–gene interaction (epistasis) across chromosomes between GABRB3 (chromosome 15) and GABRD (chromosome 1) associated with increased risk for autism.

Discussion and Conclusions

This study is, to our knowledge, the first comprehensive investigation of the allelic, genotypic, and haplotype association together with an investigation of potential gene–gene interactions of GABAR subunit genes with ASD in the Argentinean population.

As the evaluated markers did not show individual association with the disease, we performed the MDR analysis which suggested a synergistic interaction between GABRB3 and GABRD. Evidence for interaction between markers in these GABAR subunits would indicate possible epistasis between variants in these two genes in ASD cases, even when the same markers do not show individual association with the disease. The presence of an interaction effect in the absence of main effects would fit the classical definition of epistasis. A possible explanation for this finding would be that there are multiple disease variants for autism risk and that any one variant is only weakly associated [Ma et al., 2005].

Recent research indicated dysfunctional GABAR in ASD patients [Mendez et al., 2013]. GABRB3 has been associated with autism [Buxbaum et al., 2002; Cook et al.,

1998] although the findings have not been consistent across datasets [Martin et al., 2000; Salmon et al., 1999], and no reports have been published in the Argentinean population.

A significant association of GABRB3 and childhood absence epilepsy was also reported, and in silico analysis suggested that a haplotype, containing two SNPs studied here: rs4906902 and rs20317 (GABRB3), impairs binding of the neuron-specific transcriptional activator N-Oct-3 [Urak, Feucht, Fathi, Hornik, & Fuchs, 2006].

In idiopathic generalized epilepsy, a genetic variation within the GABRD gene was reported to affect GABA current amplitude, and this was suggested to be associated with increased neuronal excitability [Dibbens et al., 2004].

The frequent co-occurrence of ASD and epilepsy suggests—at least in certain cases—common neurodevelopmental bases [Brooks-Kayal, 2010]. Both ASD and epilepsy may result from common developmental pathophysiological mechanisms leading to abnormalities in connectivity, imbalance in excitation/inhibition, and disrupted synaptic plasticity.

The power of our analysis to detect genetic effects with a minor contribution, however, could be limited by the small sample size. Even so, the finding merits further investigation in a larger and/or independent sample. Clinical data from patients (clinical and cognitive symptoms associated with loss of cortical GABA-dependent inhibition) will also help evaluate an association with a subset of ASD with specific clinical and behavioral phenotypes.

The observation that 11 out of the 18 studied SNPs were monomorphic in ASD patients and controls needs further investigation to elucidate whether those polymorphisms are not present in the Argentinean population or if it is due to the sample size.

The data presented here support the hypothesis that complex interactions account for autism and may illustrate cases for complex traits, in which testing for interaction between genes uncovers a genetic liability dependent on gene interaction, even when genes involved show no independent effect.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Summary of the general steps involved in implementing the multifactor dimensionality reduction (MDR) method [adapted from Hahn et al., 2003].

Table S1. Allele frequencies of all polymorphisms in cases and controls studied in the Argentinean population ($P > 0.05$, not significant). (*) χ^2 test of overall association.

Table S2. Genotypic frequencies (cases and controls) of polymorphisms studied in the Argentinean population ($P > 0.05$, not significant). (*) χ^2 test of overall association.