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## Background

The  $\beta$ 2-adrenergic receptor (B2AR) is part of the catecholamine system, which plays a critical role in early nervous system development. Although the mature B2AR develops the mechanism of desensitization to protect against over-stimulation by ligands, fetal receptors do not have this capability (Stein, et al., 1992). Therefore, it is possible that over-stimulation of the B2AR could adversely influence CNS development, resulting in abnormalities leading to autism.

Two single nucleotide polymorphisms (SNPs) in the coding region of the B2AR gene are common in the general population and appear to produce increased receptor activity *in vivo* (Dishy et al., 2001; Bruck et al., 2003): the G (glycine) substitution at codon 16, and the E (glutamic acid) substitution at codon 27.

Connors, et al. (in press) observed, in a small sample of non-identical twins with autism, high frequencies of these two polymorphisms relative to those reported in general populations. The objective of the present study was to investigate whether the Gly16 or Glu27 alleles in the B2AR gene were associated with diagnosis of autism in the overall Autism Genetic Resource Exchange (AGRE) population.

## Methods

The population was selected from AGRE. Genotyping for rs1042713 (codon 16) and rs1042714 (codon 27) was done using TaqMan assays by design (ABI).

Allele and genotype frequencies were calculated among founders. Haplotype frequencies were estimated using an EM algorithm. Association between autism and genotype at each polymorphic site was tested using allelic and genotype-based transmission disequilibrium tests (TDT), restricting analysis to proband cases. Sensitivity to designation of the proband in each family was assessed by performing 1000 repeats of the analysis selecting probands randomly. Family based (FBAT) and haplotype based association tests (HBAT) were also conducted.

To examine possible effect modification, genotypic TDT analyses were repeated stratifying on: a) whether or not the family included an affected female; and b) presence of one or more indicators of pregnancy related stress. These indicators included report of: severe infection or fever during pregnancy, vaginal bleeding during pregnancy, generalized edema, hypertension, albuminuria, gestational diabetes, eclampsia/pre-eclampsia, multiple pregnancy, or abnormal fetal screen.

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**Table 1. Allele and genotype counts and frequencies for B2AR polymorphisms among founders (n = 753) in AGRE cohort sample. Observations total fewer than 753 due to missing values for genotypes.**

Alleles	Codon 16		Codon 27	
	Gly (G)	915 (60.8%)	Gln (C)	906 (60.2%)
Arg (A)	587 (39.0%)	Glu (G)	588 (39.0%)	
Genotypes	Arg/Arg	113 (15.0%)	Gln/Gln	268 (35.6%)
	Arg/Gly	361 (47.9%)	Gln/Glu	370 (49.1%)
	Gly/Gly	277 (36.8%)	Glu/Glu	109 (14.5%)
p-value <sup>†</sup>	<b>0.79</b>		<b>0.30</b>	

<sup>†</sup>  $\chi^2$  goodness-of-fit test for Hardy-Weinberg proportions.

**Figure 1. Estimated B2AR haplotype frequencies among founders.  $D' = 1.0$ .**

Codon 27		Codon 16	
		Arg	Gly
Gln		39%	22%
		0.1%	39%

**Table 2. Genotype frequencies among informative probands and genotype relative risk estimates for Gly16Arg and Gln16Glu B2AR polymorphisms in the AGRE cohort.**

	Cases*	Pseudo-sibs	GRR1 Designated probands	GRR2 Random probands	GRR3 Aff-male only families	GRR4 Pregnancy stress group	
<b>Number of Families</b>			358	358	238	69	
<b>Codon 16</b>	<b>Arg/Arg</b>	44	146	1	1	1	1
		18.3%	20.2%	--	--	--	--
	<b>Gly/Arg</b>	115	367	1.09	1.13	1.21	0.57
		47.7%	50.8%	(0.75 - 1.60)	(0.86 - 1.51)	(0.74 - 1.95)	(0.22 - 1.45)
	<b>Gly/Gly</b>	82	210	1.34	<b>1.48</b>	1.69	1.20
	34.0%	29.1%	(0.86 - 2.10)	(1.08 - 2.05)	(0.97 - 2.96)	(0.43 - 3.40)	
<b>Total</b>	241	723					
<b>Codon 27</b>	<b>Gln/Gln</b>	66	239	1	1	1	1
		27.2%	32.8%	--	--	--	--
	<b>Gln/Glu</b>	122	364	1.23	1.16	1.08	1.19
		50.2%	49.9%	(0.90 - 1.68)	(0.92 - 1.45)	(0.74 - 1.58)	(0.51 - 2.44)
	<b>Glu/Glu</b>	55	126	<b>1.66</b>	<b>1.47</b>	<b>1.76</b>	2.36
	22.6%	17.3%	(1.07 - 2.59)	(1.12 - 1.94)	(1.03 - 3.00)	(0.82 - 6.74)	
<b>Total</b>	243	729					

\* Informative proband cases from all families in sample.

## Results

Genotype counts and frequencies among founders are shown in Table 1 and are consistent with the literature. EM-algorithm imputed haplotype frequencies among founders are given in Figure 1; there is strong linkage disequilibrium between the two loci.

Results of genotypic TDT tests are given in Table 2. There was a statistically significant association between autism and Glu27 homozygosity. This association persisted in analyses using one randomly selected proband per family. In analyses excluding families with affected females, strength of association increased slightly. Strength of association increased more dramatically in analyses restricting to probands positive for one or more indicator of pregnancy related stress but, because of reduced sample size, did not reach statistical significance. Risk increases for Gly16 homozygosity were not consistently statistically significant.

FBAT and HBAT tests were not statistically significant; however, the deviations from expected values were in the directions consistent with the results of the TDT analyses.

## Discussion

An increased risk for autism associated with the homozygous Glu27 genotype for the B2AR gene was observed. Because the two loci studied are in strong linkage disequilibrium, separating their effects may be difficult. The Gly16 allele was also associated with an increased risk for autism, although the association was not consistently significant.

If polymorphisms in the B2AR gene increase risk for autism through a mechanism involving over-stimulation of the receptor, one would expect to observe a stronger association with genotype among groups exposed to higher levels of receptor ligands, such as terbutaline or catecholamines. We observed a higher risk for the Glu27/Glu27 genotype among the group positive for a variable constructed as a rough measure of pregnancy related stress; however, power for this aim was low and specific sources of stress, such as infection, or timing by trimester could not be investigated. Future studies including comprehensive measures of maternal stress during pregnancy for all subjects could be used to test this hypothesis.