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ORIGINAL RESEARCH ARTICLE

GAD1 (2q31.1), which encodes glutamic acid decarboxylase (GAD_{67}), is associated with childhood-onset schizophrenia and cortical gray matter volume loss

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Postmortem brain studies have shown deficits in the cortical γ-aminobutyric acid (GABA) system in schizophrenic individuals. Expression studies have shown a decrease in the major GABA-synthesizing enzyme (glutamic acid decarboxylase (GAD_{67}) mRNA levels in neurons in dorsolateral prefrontal cortex in schizophrenics relative to controls. In the present study, SNPs in and around the *GAD1* gene, which encodes the protein GAD_{67}, were tested on a rare, severely ill group of children and adolescents with childhood-onset schizophrenia (COS) ($n = 72$), in a family-based association analysis. Compared to adult-onset samples, the COS sample has evidence for more salient familial, and perhaps genetic, risk factors for schizophrenia, as well as evidence for frontal cortical hypofunction, and greater decline in cortical gray matter volume on anatomic brain MRI scans during adolescence. We performed family-based TDT and haplotype association analyses of the clinical phenotype, as well as association analyses with endophenotypes using the QTDT program. Three adjacent SNPs in the 5′ upstream region of *GAD1* showed a positive pairwise association with illness in these families ($P = 0.022$–0.057). Significant transmission distortion of 4-SNP haplotypes was also observed ($P = 0.003$–0.008). Quantitative trait TDT analyses showed an intriguing association between several SNPs and increased rate of frontal gray matter loss. These observations, when taken together with the positive results reported recently in two independent adult-onset schizophrenia pedigree samples, suggest that the gene encoding GAD_{67} may be a common risk factor for schizophrenia.

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Schizophrenia is a severe psychiatric illness, thought to be at least in part a neurodevelopmental disorder resulting in abnormalities of synaptic connectivity.[1] The illness is associated with impaired function of the cerebral cortex, particularly the prefrontal, temporal, and cingulate regions. Reduction in the inhibitory role of GABAergic neurons has been hypothesized, and postmortem studies of brains of schizophrenic patients have found changes related to inhibitory γ-aminobutyric $acid_A$ ($GABA_A$). Increased $GABA_A$ receptor binding has been reported in layers I and II of the cingulate cortex, and reduced GABA uptake and defective GABA release reported, implying that GABA transmission may be affected in schizophrenics.[2,3] Glutamic acid decarboxylase (GAD) is the key enzyme in the synthesis of GABA in inhibitory interneurons. GAD exists as two isoforms (GAD_{67} and GAD_{65}) with molecular masses of 67 and 65 kDa, which are encoded by different independently regulated genes on chromosomes 2 (*GAD1*) and 10 (*GAD2*), respectively. The two enzymes may have different physiological significance with regard to the synthesis, compartmentation, and release of GABA.[4] Both are present in most GABA-containing neurons in the CNS, but GAD_{65} appears to be targeted to membranes and nerve endings, whereas GAD_{67} is more widely distributed in cells.[5] Studies of GAD_{67} and GAD_{65} knockout mice revealed very different phenotypes: $GAD_{65}-/-$ maintained normal levels of GABA, which was in sharp contrast to $GAD_{67}-/-$ mice that died the first morning after birth and $GAD_{67}+/-$ mice that had about one-third reduction in GABA in cortex.[6,7]

A number of postmortem brain studies of schizophrenics have studied GAD expression. Akbarian *et al*[8] reported reduced prefrontal expression of

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GAD_{67} in the absence of significant cell loss; axon terminals of chandelier neurons (which are GABAergic interneurons) were reduced in the middle layers of the prefrontal cortex (PFC);[9] GAD_{67} mRNA expression was relatively unaltered in most PFC GABA neurons, but reduced below a detectable level in a subset of GABA neurons;[10] Guidotti *et al*[11] studied GAD_{67} protein expression in the PFC as well as cerebellum, and found a 50% reduction in schizophrenic and bipolar patients as compared to controls. In contrast, there were no observed differences between psychiatric and nonpsychiatric subjects in the protein levels of GAD_{65}. Taken together (though negative reports exist), these studies provide evidence supporting *GAD1* as a candidate gene in the pathogenesis of schizophrenia, and for testing *GAD2* in parallel for comparison.

Childhood-onset schizophrenia (COS), defined as the onset of psychotic symptoms by age 12, is a rare and severe form of the disorder.[12] Since 1990, patients with COS have been recruited nationally by the National Institute of Mental Health for clinical and neurobiological studies. COS appears clinically and neurobiologically continuous with the adult disorder[12] and a number of observations across pediatrics and medicine that early-onset cases may help identify a more homogeneous patient group that is less confounded by long-term effects of illness and may be caused by fewer genetic defects with higher penetrance.[13–15] Although COS is qualitatively continuous with adult-onset cases, the COS population appears quantitatively different in that there is a relatively higher rate of familial spectrum disorder,[16–18] and a higher rate of familial smooth pursuit eye movement abnormalities.[19] Further, COS is associated with poorer cognitive performance and more prominent early developmental abnormalities,[20] presumably reflecting more impaired early brain development than seen for later-onset patients. A positron emission tomography (PET) study using F-fluorodeoxyglucose (FDG) in a subset of these patients showed reduced frontal glucose metabolism similar to that seen in adult patients.[21] Finally, COS patients show a striking loss of gray matter volume as measured on serial MRI scanning through adolescence when compared with normal controls. The rate of reduction was related to premorbid impairment and baseline symptom severity,[22] and is also a greater rate of loss than reported for adult-onset cases.[23]

In order to follow-up on the positive association observed between haplotypes in the *GAD1* gene and two family samples of adult-onset schizophrenia,[24,25] we tested SNPs at this locus for association in the childhood-onset sample. A second hypothesis was that SNPs in the *GAD2* locus would not show association, consistent with expression studies. On a more exploratory level, we also tested for associations between genetic variation in *GAD1* and quantitative measures of IQ, premorbid functioning, and rate of gray matter volume loss measured on MRI scans.

Subjects and methods

Patient recruitment and clinical assessment

DNA was available for 72 children and adolescents who participated in the COS study.[12] In all, 66 had at least one parent available for study, the vast majority had both parents available ($N = 55$). A total of 42 (58%) of the COS patients were males and, given the rarity of these patients (thus precluding selection), the ethnic background (based on parental report) was quite mixed: half the sample is Caucasian, 28% is African-American, and the remaining is a mix of Hispanic (7%), Asian (5.5%) and 10% from other or mixed ethnicities. The NIMH Institutional Review Board approved the project and written consent was obtained from parents and assent from minor subjects.

Patients meeting the DSM-III-R/DSM-IV[26] criteria for schizophrenia or psychosis NOS were recruited nationwide through an extensive screening process, including a review of over 1400 charts and in-person screening of over 230 subjects. The schizophrenic patients who participated were required to have a premorbid full-scale IQ of 70 or above and an onset of psychosis by the age of 12 (average age of onset of psychotic symptoms was 10 years.) The diagnosis of COS was confirmed by two psychiatrists ($\kappa = 0.8$)[27] through an extensive evaluation that included clinical and structured interviews of the children and parents using portions of the Schedule for Affective Disorders and Schizophrenia for School-Age Children (K-SADS-E and K-SADS-PL)[28,29] and in-hospital observation during a 1–3-week medication-free period. Information on these subjects included cognitive and behavioral ratings of early development, history of medication response, neuropsychological test performance, smooth pursuit eye movements, and MRI scans.

Age of onset of first psychotic symptoms was obtained through interview and patient records. The mean age of patients when they entered our study was 14.4 years. Probands completed the Wechsler Intelligence Scale for Children-Revised (WISC-R) or Wechsler Intelligence Scale for Children-Third Edition (WISC-III). Premorbid development (defined as development up to 1 year before onset of psychosis) was evaluated based on clinical interview, neuropsychological testing, school records, standardized rating scales, and parental recall. As early-onset cases show more striking early developmental impairment,[12] ratings for early language, social, and educational adjustment were completed using a modification of the scale used by Hollis.[30] In addition, the Premorbid Adjustment Scale (PAS)[31] and the Autism Screening Questionnaire (ASQ), which has good discriminative validity with respect to the separation of Pervasive Developmental Disorder (PDD) from non-PDD diagnoses at all IQ levels,[32] were completed through parental interviews and chart review.

All patients underwent MRI scans on the same GE 1.5-T Sigma scanner during their initial in-patient

stay at NIH and at 2–8-year follow-ups (details given elsewhere).[22] Volumes of the cerebrum and total and regional gray matter were obtained by using automated analysis, and percent change in volume across time was calculated. The rate of total and frontal gray matter volume change was calculated for the subjects with COS, who had at least two MRI scans available before age 21 ($N=39$). The total gray matter slope was defined as the follow-up scan value minus the first scan value divided by time elapsed between scans and expressed as milliliters per year (a negative value representing cortical volume reduction). This measure has been presented previously (see Sporn *et al*, 2003).[22]

Nucleic acid purification and PCR

Genomic DNA was extracted from immortalized lymphoblastoid cells using the QIAamp DNA Extraction Kit (Qiagen, Inc., Valencia, CA, USA). SNPs in and around the *GAD1* and *GAD2* genes were selected from the Celera (http://www.celeradiscoverysystem.com/index.cfm) and dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) databases, and are detailed in Table 1. We used Primer Express Software (Applied Biosystems) to design *Taq*Man (fluorogenic 5′ nuclease assay) primers and probes. Reactions were performed in a 384-well format in a total reaction volume of 10 μl with 2.0 ng of dried genomic DNA, 5 μl of 2 × Ampli*Taq* Gold® PCR Master Mix (Applied Biosystems), 0.1 μl of each (1000 nM) primer, 0.02 μl of each (100 nM) probe, and 3.76 μl of 1 × TE buffer. The plates were then placed in a thermal cycler (PE 9700; Applied Biosystems) and were heated at 50°C for 2 min, then heated at 95°C for 10 min, followed by 40 cycles of 95°C for 30 s and 60°C for 1 min. Then the plates were transferred to the Prism 7900HT (Applied Biosystems), in which the fluorescence intensity in each well of the plate was read.

Statistical analysis

We carried out error checking with the program MERLIN.[33] We measured linkage disequilibrium (LD) between markers with the D' and r^2 statistics from parental haplotypes by use of the program ldmax within the GOLD software package.[34] None of the SNPs deviated from Hardy–Weinberg equilibrium. Markers were tested for transmission disequilibrium, using the TDTPHASE program of the UNPHASED software package (http://www.hgmp.mrc.ac.uk/). Analyses were carried out through the Genetic Linkage User Environment (GLUE) interface. For the TDTPHASE analysis, we ran the analysis under the 'certain haplotypes' option, which implements the ETDT method for multiallelic markers and the algorithm developed by Dudbridge[35] to avoid the potential bias introduced by uncertain phase. We tested all two-, three-, and four-marker haplotypes for association in a sliding window across the locus. The option 'drop rare haplotypes' was used to restrict the analysis to the haplotypes with a frequency $<3\%$. We carried out tests of association to quantitative traits using the program QTDT, which performed variance-components testing of family-based samples for association and transmission disequilibrium.[36] The orthogonal model used is robust to population stratification. In order to protect against possible inaccuracies due to deviations from either normality or selection on the trait, empirical *P*-values derived from 10 000 permutations are reported.

Results

We tested 14 SNPs in *GAD1* and upstream of its start site. We concentrated on the 5′ flanking region, under the assumption that, since *GAD1* expression is decreased in schizophrenic brain, at least some of the regulatory elements influencing this dysregulation will be located there. The SNP locations and allele frequencies are shown in Table 1. Also shown are the results from conventional (phase known) TDT analyses, which only counts allele transmissions from informative heterozygous parents. This analysis showed that the common allele of M06 was most strongly associated with schizophrenia, $P=0.022$. It is the SNPs in the 5′ region of *GAD1* that show a positive association with COS, whereas the remaining SNPs distal to intron 2 of the gene show no association. In contrast to these positive results in *GAD1*, none of the three *GAD2* SNPs (rs2839670, rs3781109, rs3781106) tested showed a positive association in these families, $P=0.37$–0.79 (data not shown, details available upon request).

We computed marker-to-marker LD separately using the Caucasian and African-American subsamples. There was moderate LD between all markers throughout the *GAD1* region in Caucasians, but this was not as clear in the African-American sample (see Table 2). This observation is consistent with the knowledge that LD between closely spaced markers tends to be less strong in African populations.[37] The haplotypes that encompass the 5′ transcription start site and the protein initiator codon showed transmission distortion to schizophrenics. The detailed results of the only significantly associated four-marker haplotype, which contained markers M04–M07, is shown in Table 3. We observed three common haplotypes accounting for 98% of the variation. The most common haplotype (65%)) was overtransmitted to affecteds ($P=0.005$).

A total of 30 quantitative intermediate phenotypes, including age of onset, 14 cognitive measures from the WISC (including IQ), 12 premorbid measures (described in the Subjects and methods section), eye tracking, and longitudinal gray matter loss, were tested for association with all 14 *GAD1* SNPs. This QTDT analysis revealed that six out of 10 SNPs tested showed an association with more marked cortical gray matter loss over time, $P=0.04$–0.003. Four SNPs, M03, M05, M11, and M13, were not testable due to the small number of informative transmissions. In addition, M03 was associated with a poor qualitative eye-tracking score, $P=0.003$, thought to reflect

Table 1 Marker and map information and single SNP TDT results

M#	SNP-ID	Coding strand SNP[a]	Allele 2 frequency[b]	Location	Chr. 2 position July 2003 UCSC freeze	Intermarker distance (bp)	Distance from M01	Entire COS sample T/NT[c]	Entire COS sample P-value	Female offspring only T/NT[c]	Female offspring only P-value
M01	hCV2177469	G/A	0.35	5′ Flanking	171862856	0	0	27/20	0.306	12/9	0.512
M02	hCV2177464	T/C	0.23	5′ Flanking	171867869	5013	5013	22/14	0.181	10/4	0.103
M03	hCV11637130, rs1978340	G/A	0.25	5′ Flanking	171872665	4796	9809	12/19	0.207	5/10	0.192
M04	rs872123	T/C	0.34	5′ Flanking	171873710	1045	10854	21/13	0.168	10/4	0.103
				Start site of transcript NM_000817:	171875616						
M05	hCV2177452, rs3749034	G/A	0.15	Exon 1 (5′ UTR)	171876019	2309	13163	16/7	0.057	9/2	0.028
M06	rs2270335	C/T	0.32	Intron 1	171877240	1221	14384	23/10	0.022	12/3	0.016
				ATG (Met):	171877646-48						
M07	rs2241165	A/G	0.35	Intron 2	171880923	3683	18067	24/12	0.043	12/3	0.016
M08	hCV8823462, rs769404	T/C	0.40	Exon 3 (His37His)	171881169	246	18313	21/27	0.386	11/13	0.683
M09	hCV2177441, rs3828275	C/T	0.38	Intron 3	171885284	4115	22428	23/26	0.668	11/14	0.548
M10	hCV2177434	C/G	0.23	Intron 5	171891137	5853	28281	16/14	0.715	8/5	0.403
M11	rs769390	A/C	0.20	Intron 6	171895999	4862	33143	15/16	0.857	7/11	0.344
M12	hCV8823482, rs701492	C/T	0.30	Intron 9	171905024	9025	42168	20/19	0.873	5/11	0.129
M13	rs3791850	G/A	0.13	Intron 12	171910644	5620	47788	12/5	0.085	6/1	0.047
M14	hCV8823522, rs769395	A/G	0.28	Exon 17 (3′ UTR)	171919347	8703	56491	18/19	0.869	9/11	0.655

[a]Common allele listed first.
[b]Allele frequencies computed from parental genotypes.
[c]Transmissions of common alleles reported.
T = transmitted; NT = not transmitted.
Phase-known analysis counts the number of transmitted and nontransmitted alleles to those affected from heterozygous parents.

Table 2 Marker-to-marker LD in the *GAD1* locus

SNP	*M01*	*M02*	*M03*	*M04*	*M05*	*M06*	*M07*	*M08*	*M09*	*M10*	*M11*	*M12*	*M13*	*M14*
Caucasian (number founders = 121)														
M01		0.78	0.19	0.64	0.65	0.59	0.61	0.04	0.07	0.42	0.09	0.10	0.43	0.09
M02	1.00		0.15	0.85	0.86	0.78	0.79	0.14	0.17	0.59	0.06	0.07	0.59	0.06
M03	1.00	1.00		0.16	0.14	0.16	0.15	0.32	0.14	0.13	0.58	0.52	0.11	0.55
M04	0.89	0.94	1.00		0.87	0.91	0.94	0.19	0.23	0.68	0.06	0.08	0.66	0.07
M05	0.96	0.96	1.00	1.00		0.89	0.93	0.21	0.23	0.74	0.05	0.06	0.81	0.06
M06	0.85	0.90	1.00	0.97	1.00		0.97	0.18	0.26	0.69	0.06	0.08	0.67	0.07
M07	0.86	0.90	1.00	0.97	1.00	1.00		0.22	0.26	0.74	0.06	0.08	0.71	0.07
M08	0.36	0.80	1.00	0.90	1.00	0.89	1.00		0.71	0.16	0.24	0.26	0.21	0.24
M09	0.48	0.83	0.64	0.92	1.00	1.00	1.00	0.89		0.23	0.27	0.30	0.23	0.28
M10	0.75	0.78	1.00	0.85	0.88	0.88	0.89	0.88	1.00		0.10	0.12	0.89	0.11
M11	0.76	0.71	0.82	0.72	0.69	0.72	0.72	0.93	0.94	1.00		0.91	0.10	0.97
M12	0.79	0.74	0.76	0.75	0.70	0.74	0.75	0.93	0.94	1.00	1.00		0.13	0.89
M13	0.78	0.81	1.00	0.88	0.96	0.88	0.89	1.00	1.00	0.96	1.00	1.00		0.11
M14	0.78	0.73	0.79	0.72	0.70	0.72	0.72	0.93	0.94	1.00	1.00	0.97	1.00	
African-American (number founders = 36)														
M01		0.29	0.12	0.13	0.07	0.13	0.17	0.04	0.06	0.09	0.08	0.02	0.06	0.29
M02	1.00		0.02	0.04	0.24	0.04	0.03	0.00	0.06	0.25	0.02	0.01	0.05	0.07
M03	1.00	0.76		0.31	0.00	0.30	0.31	0.04	0.05	0.10	0.17	0.16	0.01	0.28
M04	0.57	0.55	1.00		0.03	0.93	0.94	0.44	0.29	0.01	0.21	0.06	0.02	0.06
M05	1.00	1.00	1.00	1.00		0.03	0.02	0.01	0.02	0.08	0.01	0.00	0.32	0.01
M06	0.62	0.59	1.00	1.00	1.00		1.00	0.44	0.30	0.04	0.22	0.08	0.02	0.03
M07	0.69	0.54	1.00	1.00	1.00	1.00		0.44	0.31	0.02	0.23	0.08	0.01	0.05
M08	0.52	0.31	1.00	1.00	1.00	1.00	1.00		0.49	0.00	0.09	0.00	0.01	0.00
M09	0.57	1.00	1.00	0.75	1.00	0.74	0.75	0.77		0.00	0.00	0.12	0.01	0.14
M10	0.33	0.84	1.00	0.16	1.00	0.33	0.22	0.01	0.04		0.07	0.17	0.13	0.27
M11	1.00	1.00	0.47	1.00	1.00	1.00	1.00	0.41	0.09	1.00		0.30	0.01	0.27
M12	0.26	0.37	0.61	0.28	0.05	0.34	0.31	0.01	1.00	0.80	1.00		0.03	0.24
M13	1.00	0.49	1.00	0.99	1.00	1.00	0.96	1.00	1.00	1.00	1.00	1.00		0.03
M14	1.00	0.98	0.82	0.26	1.00	0.20	0.25	0.17	1.00	1.00	1.00	0.53	1.00	

Correlation coefficient r^2 above diagonal, Lewontin's D' below.

impaired frontal lobe function. Further, we observed a positive association between M03 and M08 and the motor component of the Hollis scale ($P = 0.011$ and 0.030, respectively). Finally, M03 was associated with the total score on the Hollis scale, $P = 0.016$, reflecting premorbid impairment in domains covering school, social, language, and motor development. In all cases, the alleles that were positively associated with the clinical phenotype were those associated with poorer scores on these quantitative measures. In contrast, none of the cognitive measures assessed by the WISC showed any association with any of the SNPs. All of the QTDT results are shown in Supplementary Table 1.

Table 3 Four-marker haplotype results

Haplotype		*Phase known*			
M04-M07	*Freq*	*T*	*NT*	*P-value*	*RR*
T-G-C-A	0.65	17	4	0.005	1.33
C-A-T-G	0.16	1	11	0.003	0.14
C-G-T-G	0.17	3	4	0.008	1.00
Global[a]				0.009	

[a]Computed by likelihood ratio test, dropping rare haplotypes with frequency <3%: LRS = 11.64; df = 3; $P = 0.009$.
T = transmitted; NT = not transmitted; RR = relative risk.
Phase-known analysis counts the number of transmitted and nontransmitted haplotypes to those affected from unambiguous parental matings.

Discussion

We observed a positive association between SNPs in the *GAD1* locus, which encodes for the major GABA-synthesizing enzyme GAD_{67}, and COS. The three SNPs that showed the strongest associations, M05, M06, and M07, are located in the 5′ upstream region of the gene, from exon 1 (which is all 5′ UTR), and in introns 1 and 2, respectively. This is perhaps not surprising, given the downregulation of the GAD_{67} transcripts and protein observed in the PFC of schizophrenics. If these SNPs are tagging a disease-associated haplotype for schizophrenia, they may lead to a change in the expression level of *GAD1* in

some tissues and/or under certain conditions. Analysis through MatInspector of Genomatix showed that replacing G with A at rs3749034 creates two additional putative transcription factor (TF) binding sites—namely ATP1a1 regulatory element binding factor 6 (AREB6) and myoblast determining factor (MYOD), while changing T to C at rs2270335 increases one more prediction of TF-binding site, that is, PAX5. Both AREB6 and PAX5 have been shown to have various effects on gene transcriptions,[38,39] though it remains to be seen if they are functional in the *GAD1* promoter region. An alternative possibility is that these SNPs are in LD with other polymorphisms that affect transcription and/or splicing. In addition, these same SNPs and alleles showed a significant association with gray matter loss over time. Specifically, the common allele that was overtransmitted to affecteds was also associated with increased frontal gray matter volume loss on the MRI measures. This is a intriguing finding that deserves follow-up, particularly in light of the fact that we had multiple volumetric scans on only 39 of our cases for analysis. Moreover, other genes that we studied (eg G72, DTNBP1)[40,41] did not show such an association, suggesting that this finding is not an artifact/confound under the diagnosis of COS. Finally, as we expected, there was no association observed between SNPs in the *GAD2* locus, which encodes for GAD_{65}.

Comparison of the findings in this study with the recent observations of association between *GAD1* and two independently collected adult-onset samples is somewhat complicated by the fact that in the original work that we based our study on, they observed positive associations with different SNPs and haplotypes in each of their samples.[24,25] In addition, in one of the two samples, they observed a significant association with distorted transmission only to female patients. Interestingly, when we subdivided our analysis by gender, the signal is similarly stronger in the females. Further, the haplotype that we observed to be significantly overtransmitted in the COS sample was characterized by the same common alleles as the significant haplotypes reported in the CBDB Sibling Study sample.[24,25] One other paper by De Luca *et al*[42] recently reported a negative association with both the *GAD1* and *GAD2* genes, but those researchers tested only one SNP in each gene, which may have led to a false-negative result. In fact, the one SNP in GAD1 that they tested, rs769404, was also negative in the current study.

Overall, the results of the endophenotype analyses indicated a relationship between variation in the *GAD1* gene and an increased rate of frontal gray matter volume loss on MRI, and an association with eye-tracking deficits in a single but different SNP. While eye-tracking deficits may involve frontal lobe function,[43] other measures of prefrontal function (eg working memory subscales of the WISC, eg digit span) did not show association. While most of the SNPs tested revealed a positive association with increased frontal gray matter volume loss on MRI over time, one SNP, M03, also showed a positive association with eye-tracking dysfunction and a measure of premorbid functioning, which covers areas such as school, social, speech, and motor delays and difficulties. Given that the markers are in partial LD with each other and many of the phenotypic measures are also strongly correlated, the tests are not independent and therefore a Bonferroni correction would not be appropriate. We are not aware of a straightforward way to adjust for multiple testing in this situation, and recognize that type I errors are certainly cause for concern. We therefore present these endophenotype results as preliminary, but deserving of further study by other case–control and family samples. Nonetheless, we note that in our studies of three schizophrenia susceptibility genes to date, each gene demonstrates a very distinct pattern of association with the endophenotypic measures. Specifically, the G72 gene was associated with age of onset and scores on the Autism Screening Questionnaire,[40] while SNPs in the gene Dysbindin were associated with scores on the Premorbid Development Scale.[41] It is plausible that careful study of quantitative intermediate phenotypes in relationship to susceptibility genes for complex diseases such as schizophrenia can elucidate characteristic effects of each gene and how it may relate to the disease of interest.

As with most studies of rare diseases, many limitations exist. The children selected for this study were nationally recruited for an inpatient clinical trial and unknown referral bias is likely (eg more complete families, ability to travel, less ill parents). This group consisted of severely ill, treatment refractory subjects, and thus may not be representative of early-onset subjects in general. In addition, the small size of the sample greatly limits the power to detect associations, thus many of the SNPs that did not demonstrate association with either the clinical or endophenotypes may eventually do so in larger samples, and such efforts are now underway by at least two other research groups. Nonetheless, even given limited power, and the conservative TDT test utilized in this study, the fact that we did observe a strong association with SNPs in the *GAD1* gene indicates that this gene may have a relatively large effect in this sample (relative risks = 2.0–2.3). Studies of very early onset population with more pronounced neurobiological abnormalities and a more homogeneous phenotype may well turn out to be a relatively efficient population for the identification of some risk genes. Further strength for this argument is also provided by our recent report of an association with the G72 risk gene.[40]

In conclusion, as might have been expected from numerous studies of dysfunctional regulation of molecules in the GABAergic system in the brains of schizophrenics, a family-based analysis showed that polymorphisms in the 5′ upstream region of *GAD1* were positively associated with COS and cortical gray matter loss over time. These observations, when taken together with the postmortem expression studies and

the positive results obtained from two independent adult-onset schizophrenia pedigree samples,[24,25] suggest that the gene encoding GAD_{67} may prove to be a fairly common genetic risk factor for schizophrenia.

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