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Investigation of the G protein subunit $G\alpha_{olf}$ gene (*GNAL*) in attention deficit/hyperactivity disorder

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Abstract

The dopamine system plays an important role in the regulation of attention and motor behavior, subsequently, several dopamine-related genes have been associated with Attention Deficit/Hyperactivity Disorder (ADHD). Among them are the dopamine receptors D1 and D5 that mediate adenylyl cyclase activation through coupling with G_s-like proteins. We thus hypothesized that the G_s-like subunit $G\alpha_{olf}$, expressed in D1-rich areas of the brain, contributes to the genetic susceptibility of ADHD. To evaluate the involvement of the $G\alpha_{olf}$ gene, *GNAL*, in ADHD, we examined the inheritance pattern of 12 *GNAL* polymorphisms in 258 nuclear families ascertained through a proband with ADHD (311 affected children) using the transmission/disequilibrium test (TDT). Categorical analysis of individual marker alleles demonstrated biased transmission of one polymorphism in *GNAL* intron 3 (rs2161961; $P = 0.011$). We also observed significant relationships between rs2161961 and dimensional symptoms of inattention and hyperactivity/impulsivity ($P = 0.003$ and $P = 0.008$). In addition, because of recent evidence of imprinting at the *GNAL* locus, secondary analyses were split into maternal and paternal transmissions to assess a contribution of parental effects. We found evidence of strong maternal effect, with preferential transmission of maternal alleles for rs2161961A ($P = 0.005$) and rs8098539A ($P = 0.035$). These preliminary findings suggest a possible contribution of *GNAL* in the susceptibility to ADHD, with possible involvement of parent-of-origin effects.

Keywords

Attention deficit/hyperactivity disorder; Genetics; G protein; $G\alpha_{olf}$; Transmission/disequilibrium test; Dopamine receptor D1

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1. Introduction

Attention Deficit/Hyperactivity Disorder (ADHD) is a common neurodevelopmental condition characterized by a pattern of inattention, hyperactivity and impulsivity. Current hypotheses on the biological basis of ADHD have centered on the dysregulation of fronto-striatal circuits and the neurotransmitters involved in these pathways. In particular, accumulating evidence implicate altered dopamine signalling in the disorder (Davids et al., 2003; Durston, 2003; Seeman and Madras, 1998; Viggiano et al., 2003) and genetic association of several genes engaged in dopamine signalling is supported by meta-analysis of pooled data (e.g. *DRD4*, *DRD5*, *DAT1*, and *SNAP25*) (Thapar et al., 2005).

We previously reported the association between the dopamine receptor D1 gene (*DRD1*) and ADHD (Misener et al., 2004), particularly between one haplotype and inattention symptoms ($P = 0.008$). Recently, we replicated the association between this haplotype and inattentive behaviors in children selected for reading difficulties ($P = 0.004$) (Luca et al., submitted for publication). Positive findings were also found for one *DRD1* marker in an ADHD case-control sample (Bobb et al., 2005), although negative results were also obtained with smaller family-based samples for single markers (Bobb et al., 2005; Kirley et al., 2002). Our findings for *DRD1* in ADHD symptoms are suggestive of a potential role of the D1/D5 signalling pathways in genetic susceptibility of this disorder. This is further supported by a large combined analysis of 14 independent samples of 1980 probands ($P = 0.00005$), odds ratio 1.24 (Lowe et al., 2004) for *DRD5* (a D1-like receptor). In the same vein, we have recently reported evidence of association between ADHD and the calcyon gene, a D1-interacting protein (Laurin et al., 2005).

D1/D5 signalling mediates executive abilities including working memory (Goldman-Rakic et al., 2000), attention (Bayer et al., 2000; Granon et al., 2000), motor control (Dreher and Jackson, 1989; Meyer, 1993), and reward and reinforcement mechanisms (Beninger and Miller, 1998). Impairment of those functions is often observed in individuals with ADHD (Arnsten and Li, 2005b; Lijffijt et al., 2005; Luman et al., 2005; Martinussen et al., 2005; Willcutt et al., 2005). Moreover, a recent study in rodents suggested that D1 stimulation contributes to cognitive-enhancing effects of methylphenidate, a leading treatment for ADHD (Arnsten and Dudley, 2005a).

D1 signalling is mediated in the brain by the heterotrimeric G proteins G_s and G_{olf} (Corvol et al., 2001; Zhuang et al., 2000), which cause activation of adenylyl cyclase, cAMP-dependant protein kinase, and DARPP32. D1 receptors also signal via phospholipase C-dependent mobilization of intracellular calcium (Undie and Friedman, 1990; Wang et al., 1995), likely involving calcyon (Lezcano et al., 2000). Lesion experiments and knockout studies have indicated that the coupling of D1 receptors to adenylyl cyclase is mostly provided by $G\alpha_{olf}$ in the striatal neurons, and that $G\alpha_{olf}$ is required for D1-mediated behaviour and biochemical effects in the striatum (Corvol et al., 2001; Herve et al., 1993; Zhuang et al., 2000). $G\alpha_{olf}$ appears to be highly regulated by receptor usage and availability of interacting/effector proteins (Corvol et al., 2004, 2001; Herve et al., 2001, 1993; Iwamoto et al., 2004; Schwindinger et al., 2003; Zhuang et al., 2000), suggesting that it represents a limiting factor in the coupling efficiency of D1 receptors.

Based on our previous finding for *DRD1* in ADHD symptoms and the regulatory role played by $G\alpha_{olf}$ in D1 signalling, we believe that the $G\alpha_{olf}$ gene, *GNAL*, is a reasonable candidate for involvement in ADHD susceptibility. This is further supported by the locomotor behaviour of the mice deficient for $G\alpha_{olf}$. When tested in open field exercises, the *GNAL*+/− mice exhibit a slight decrease in basal locomotor activity, while the −/− mice display locomotor hyperactivity (Belluscio et al., 1998; Schwindinger et al., 2003) similar to a D1 knockout (Xu et al., 1994a,b).

The *GNAL* gene is located on the short arm of chromosome 18 in a region that has been linked to bipolar disorder and schizophrenia (Berrettini, 2000; Schwab et al., 2000; Segurado et al., 2003), with some evidence of parent-of-origin effects (Gershon et al., 1996; Nothen et al., 1999; Stine et al., 1995). However, replication studies have led to conflicting results (Van Broeckhoven and Verheyen, 1998; Zill et al., 2003).

In the present study, we sought evidence for association between *GNAL* and ADHD in a sample of clinically ascertained nuclear families. We tested for the non-random transmission of alleles of 12 single nucleotide polymorphisms (SNPs) using the transmission/disequilibrium test (TDT) statistic (Spielman and Ewens, 1996). Given previous findings suggesting parent-of-origin effects at 18p and evidence of epigenetic modification of *GNAL* (Corradi et al., 2005), we also assessed transmissions from mothers and fathers separately. Finally, we performed quantitative analysis using ADHD inattentive and hyperactive/impulsive symptom counts.

2. Materials and methods

2.1. Study sample and diagnostic assessment

The methods of assessment, characteristics of the subjects, and inclusion/exclusion criteria have been described previously, including the instruments used to collect information for the diagnosis of ADHD and co-morbid conditions (Barr et al., 1999; Laurin et al., 2005; Quist et al., 2000). Briefly, probands and their siblings between 7 and 16 years old were included if they met DSM-IV criteria for one of the three ADHD subtypes. The study sample was comprised of 258 nuclear families from the Toronto area, including 53 affected siblings. This gave a total of 311 affected children (251 boys and 60 girls) that were genotyped along with 209 fathers and 243 mothers. The sample consists of 194 parents-child trios and 64 families in which a single parent was genotyped. The distribution of the affected children among the DSM-IV ADHD subtypes was 14% of the predominantly hyperactive/impulsive subtype, 24% of the predominantly inattentive subtype and 62% of the combined subtype. All children were free of medication for 24 h before assessment. This protocol was approved by the Hospital for Sick Children's Research Ethics Board and informed written consent or verbal assent (children) was obtained for all participants.

Information on ADHD symptoms was obtained using semi-structured interviews for parents (Parent Interview for Child Symptoms: PICS-IV; Ickowicz et al., 2006) and teachers (Teacher Telephone Interview: TTI-IV; Tannock et al., 2002). These instruments were used to determine symptom scores based on the nine DSM-IV criteria for both inattention and hyperactivity/impulsivity dimensions. In our study sample, parent-reported symptom scores

range from 0 to 9 for both hyperactive/impulsive (mean = 5.54 ± 2.34) or inattentive (mean = 5.85 ± 1.99) behavior. The corresponding teacher-reported scores also range from 0 to 9 (mean = 4.17 ± 2.78 and 5.22 ± 2.21, respectively).

2.2. Genotyping

DNA was extracted from blood lymphocytes using a standard high salt extraction method (Miller et al., 1988). A total of 12 single nucleotide polymorphisms (SNPs) were genotyped using the ABI 7900-HT Sequence Detection System® (Applied Biosystems) and TaqMan 5′ nuclease assays for allelic discrimination (Livak, 1999). Primer and probe sequences are available on request. The PCR reactions (5 μl) contained 30 ng of genomic DNA, 10 μM of Taq-Man® Universal PCR Master Mix and 0.1 μl of allelic discrimination mix (Applied Biosystems). The thermal cycling conditions were 95 °C for 10 min and 40–60 cycles of 95 °C for 15 s and the annealing temperature (58–60 °C) for 1 min.

2.3. Statistical analysis

The TDTPHASE and PDTPHASE programs from the UNPHASED package v2.403 (Dudbridge, 2003) were used to test for biased transmission of individual marker alleles in relation to ADHD diagnosis (categorical analysis). TDTPHASE was used to test transmissions from mothers and fathers separately. Because 53 affected siblings were included in our study, we also provided the results of the TDT analysis using one randomly chosen sibling per family as well as examined the results using PDT, an extension of the TDT, which provides a valid test of association in the presence of linkage in families with multiple affected siblings and thus, is suitable for both family trios and sibling pair structures. Quantitative trait TDT analyses, examining the transmission of individual alleles in relation to inattentive and hyperactive/impulsive symptom scores were carried out using the FBAT program v1.5.5 (Horvath et al., 2001), with the additive model of inheritance. For the tests, we used population-based mean scores as an offset value to mean centre the trait. The coefficients of linkage disequilibrium (LD) between marker alleles, Δ^2 and D', were calculated using Haploview v2.03 (Barrett et al., 2005). Two-sided *P*-values were used for all results. Permutation testing was performed for TDTPHASE and PDTPHASE analyses. The corrected values for the most significant *P* values are reported together with the uncorrected *P* values. Our analysis of quantitative trait is not corrected for multiple tests as these are secondary analyses based on our findings from the categorical analyses.

3. Results

In this study, we selected a total of 12 non-coding SNPs across the *GNAL* gene to test for association between the *GNAL* gene and ADHD. Their location within the gene is presented in Fig. 1. Markers with high heterozygosity were initially chosen across the gene and additional markers were added to follow up positive findings. We included two SNPs described previously by Berrettini et al. (1998) in *GNAL* introns 3 (rs8095592) and 10 (rs3892113). Pairwise measures of linkage disequilibrium between adjacent SNPs are shown in Table 1. Parental allele frequencies are shown in Table 2. The allele frequencies of rs8095592 (*G* = 70%; *A* = 30%) and rs3892113 (*T* = 92%; *G* = 8%) are different from the ones reported by Berrettini et al. in a control sample drawn from an American population

(rs8095592 G = 31%, A = 69%; and rs3892113 T = 84%, G = 16%), but were similar to the ones reported by Zill et al. (2002, 2003) in European controls (rs8095592 G = 69–72%, A = 28–31%; and rs3892113 T = 88–94%, G = 6–12%). We confirmed the allele identity of these two markers in our sample by sequencing. Genotypes demonstrated no significant departure from Hardy–Weinberg equilibrium.

Categorical analysis revealed allelic association for rs2161961 (Table 2). Allele A was transmitted 101 times and untransmitted 68 times ($P_{TDT(all)}$=0.011;$P_{TDT(one\ sib)}$= 0.005; P_{PDT} = 0.013). The other 11 markers failed to show a significant association (Table 1), although a weak trend was observed for rs8098539 ($P_{TDT(all)}$ = 0.081; $P_{TDT(one\ sib)}$ = 0.111; P_{PDT} = 0.096), located 886 bp downstream and demonstrating strong, although not complete, LD with rs2161961 (D' = 0.986, Δ^2 = 0.583). When the results for rs2161961 were corrected for the total number of markers tested the results were no longer significant, although a trend was still evident (P = 0.071–0.101).

We also performed secondary analyses to examine the relationship of this gene to symptom counts of inattention or hyperactivity/impulsivity. Twin studies indicate that there are shared as well as independent genes contributing to the symptom dimensions of ADHD (Levy et al., 1997), thus we examine the genetic contribution to the dimensions separately. The marker rs2161961 showed significant association to both ADHD dimensions as rated by the parent (inattention: P = 0.003 and hyperactivity/impulsivity: P = 0.008) (Table 3).

As mentioned previously, genetic linkage studies for bipolar disorder and schizophrenia have suggested parent-of-origin effects for the chromosome 18p region encompassing *GNAL*. Furthermore, molecular studies assessing methylation status of the two promoters of the gene suggest that *GNAL* is subject to epigenetic regulation (Corradi et al., 2005). We thus examined the data for the presence of a parent-of-origin effect (Table 4). When assessing transmission separately for mothers and fathers, mothers appeared to transmit preferentially the rs2161961A allele (P = 0.005), as well as the rs8098539A alleles (P = 0.035). No bias was observed for paternal transmission of the same alleles (P = 0.271 and P = 0.502, respectively).

4. Discussion

In this study, we investigated the involvement of the G protein subunit α_{olf} gene, *GNAL*, in ADHD. Our TDT analysis of 12 SNPs spanning *GNAL* in a clinically ascertained sample revealed the nominally significant association of rs2161961 alleles with ADHD diagnosis as well as with quantitative traits of inattention and hyperactivity/impulsivity as rated by parents. Moreover, we observed that these findings were most likely attributable to an excess of maternal transmissions.

$G\alpha_{olf}$ is a major regulatory target in D1 signalling and may modulate the intensity of the D1 response. The importance of the balance in D1 signalling in the brain has been highlighted by the demonstration that excessive as well as insufficient D1 receptor stimulation impairs prefrontal cognitive function (Arnsten and Li, 2005b; Williams and Castner, 2006). ADHD is a complex disorder that occurs across the developmental spectrum, with the course of the

illness changing over time. Maturational changes in the dopamine system, particularly in D1 signalling, have been suggested to be involved in the emergence and course of ADHD (Andersen and Teicher, 2000; Diaz Heijtz et al., 2004). Interestingly, studies in rodents have shown that the levels of the $G\alpha_{olf}$ protein vary differentially across the developmental course in the striatum (Rius et al., 1994).

Pleiotropic effects of a susceptibility gene at 18p11.2 has been suggested by evidence of linkage in a proportion of families with bipolar disorder, recurrent major depression and/or schizophrenia (Balciuniene et al., 1998; Berrettini, 2000; Schwab et al., 2000; Segurado et al., 2003). Those findings are, however, not universal (Badner and Gershon, 2002; Lewis et al., 2003; Van Broeckhoven and Verheyen, 1999), and their interpretation is further complicated by the putative presence of a parent-of-origin effect in this region (Petronis, 2000). Stronger evidence of linkage has been reported in this region for bipolar families with *paternal* transmission of the phenotype (Gershon et al., 1996; Nothen et al., 1999; Stine et al., 1995), and with *maternal* pedigrees for schizophrenia (Schwab et al., 1998). The investigation of *GNAL* as a candidate gene in this region for both bipolar disorder (Tsiouris et al., 1996; Turecki et al., 1996; Zill et al., 2003) and major depression (Zill et al., 2002) yielded negative results. However, only one or two markers were assessed in each of these studies. To our knowledge, this is the first time that *GNAL* was investigated in relation to ADHD. Our findings of biased transmission of maternal, but not paternal, alleles of rs2161961 and rs8098539, are consistent with the presence of parental effect and with genomic imprinting of the *GNAL* gene region (Corradi et al., 2005).

$G\alpha_{olf}$ is a key effector molecule in signalling of the D1 receptor, for which evidence of association has been reported for ADHD (Bobb et al., 2005;Misener et al., 2004), particularly with the inattention symptoms in children with ADHD (Misener et al., 2004) or reading difficulties (Luca et al. submitted for publication). Most association studies have focused on neurotransmitter receptor or transporter level, however, G proteins are central in the regulation of different pathways. The findings of this study provide some support for the involvement of $G\alpha_{olf}$ in susceptibility to ADHD. We acknowledge, however, that these are preliminary results that require replication in independent samples, particularly in light of the number of markers tested for this gene as well as for other genes in this sample.

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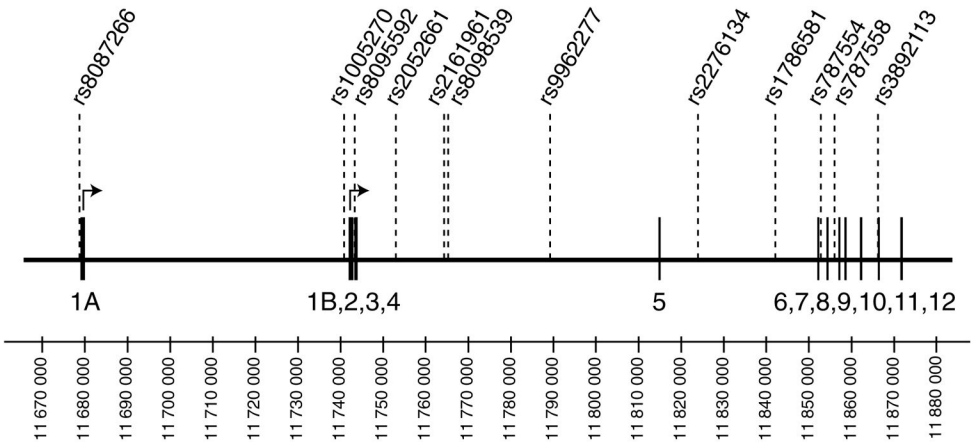

Fig. 1.

Schematic representation of the *GNAL* gene and location of the genotyped polymorphisms within the gene. Exons are indicated by boxes and markers are shown above the gene. Two major alternative transcription start sites have been described for *GNAL*, giving rise to isoforms that differ in their first exons (1A or 1B), but share exons 2 to 12 (Corradi et al., 2005; Vuoristo et al., 2000).

Table 1

Pairwise linkage disequilibrium relationships (D' and Δ^2)[a] between the *GNAL* markers[b,c]

	7266	5270	5592	2661	1961	8539	2277	6134	6581	7554	7558	2113
7266		0.045	0.204	0.203	0.253	0.376	0.130	0.109	***0.679***	0.116	0.190	0.061
5270	0.001		**0.912**	***0.767***	***0.743***	***0.724***	***0.745***	***0.776***	0.330	0.319	0.306	0.019
5592	0.041	0.384		**0.887**	**0.862**	**0.872**	**0.801**	**0.812**	*0.557*	0.317	0.391	0.067
2661	0.041	0.276	***0.787***		**0.895**	**0.908**	**0.844**	**0.848**	***0.652***	0.350	*0.427*	0.281
1961	0.050	0.330	*0.580*	***0.639***		**0.986**	**0.886**	**0.880**	**0.967**	0.234	0.341	0.294
8539	0.108	0.184	*0.574*	***0.630***	*0.583*		**0.973**	**0.889**	**0.933**	0.338	*0.402*	0.028
2277	0.010	*0.417*	0.390	*0.439*	***0.621***	*0.437*		**0.894**	**0.916**	0.237	0.238	0.050
6134	0.005	*0.530*	0.270	0.298	*0.406*	0.247	*0.519*		**1.000**	*0.415*	*0.414*	***0.620***
6581	0.038	0.004	0.026	0.035	0.061	0.094	0.042	0.034		*0.553*	***0.609***	0.033
7554	0.009	0.072	0.068	0.084	0.048	0.058	0.051	0.105	0.017		**0.960**	*0.587*
7558	0.031	0.050	0.133	0.160	0.105	0.107	0.039	0.080	0.027	***0.705***		***0.611***
2113	0.000	0.000	0.001	0.003	0.003	0.000	0.000	0.006	0.000	0.009	0.012	

[a] D' and Δ^2 values are given in the top right and bottom left of the table, respectively.

[b] SNP IDs are abbreviated to the final four digits.

[c] Bold for D' or Δ^2 values >0.80, bolditalics for values between 0.80 and 0.60, italics for 0.59–0.40.

Table 2

Transmission disequilibrium test for individual *GNAL* polymorphisms

SNP	Allele	Freq.	Transmissions		$TDT_{(all)}$		$TDT_{(one\ sib)}$		PDT	
			T	NT	χ^2	*P*	χ^2	*P*	*Z*	*P*
rs8087266 G/A	G	0.70	90	70	2.507	0.113	2.355	0.125	1.787	0.074
rs1005270 T/C	T	0.83	77	67	0.695	0.405	1.526	0.217	0.953	0.341
rs8095592 G/A	G	0.70	98	87	0.654	0.419	0.530	0.467	0.550	0.583
rs2052661 G/C	G	0.71	96	81	1.273	0.259	0.961	0.327	0.999	0.318
rs2161961 A/G	A	0.75	101	68	6.485	0.011*	7.796	0.005*	2.494	0.013*
rs8098539 A/G	A	0.64	107	83	3.040	0.081	2.538	0.111	1.665	0.096
rs9962277 C/T	C	0.79	73	64	0.592	0.442	1.072	0.300	0.752	0.452
rs2276134 T/C	T	0.85	55	54	0.009	0.924	0.045	0.831	−0.157	0.876
rs1786581 C/T	C	0.83	71	61	0.758	0.384	0.154	0.695	1.021	0.307
rs787554 C/T	C	0.78	72	88	1.603	0.206	0.954	0.329	−1.265	0.206
rs787558 T/C	T	0.73	93	81	0.828	0.363	1.363	0.243	1.025	0.305
rs3892113 T/G	T	0.92	32	29	0.148	0.701	0.308	0.579	0.000	1.000

** P* value after 1000 permutations : $P_{TDT(all)} = 0.101$; $P_{TDT(one\ sib)} = 0.071$; $P_{PDT} = 0.101$.

Table 3

Quantitative analysis of *GNAL* allele transmission in relation to ADHD symptom scores reported by parents (PICS-IV) and teacher (TTI-IV)

Marker	Allele	Informative families	Inattention symptoms				Hyperactivity/impulsivity symptoms			
			Parent (PICS-IV)		Teacher (TTI-IV)		Parent (PICS-IV)		Teacher (TTI-IV)	
			Z	*P*-value	*Z*	*P*-value	*Z*	*P*-value	*Z*	*P*-value
rs8087266	G	101	1.712	0.087	1.316	0.188	1.767	0.077	1.655	0.098
	A		−1.712	0.087	−1.316	0.188	−1.767	0.077	−1.655	0.098
rs1005270	T	85	1.141	0.254	0.116	0.908	0.133	0.895	0.024	0.981
	C		−1.141	0.254	−0.116	0.908	−0.133	0.895	−0.024	0.981
rs8095592	G	110	1.373	0.170	0.020	0.984	0.241	0.809	−0.168	0.866
	A		−1.373	0.170	−0.020	0.984	−0.241	0.809	0.168	0.866
rs2052661	G	109	1.345	0.179	0.083	0.934	0.863	0.388	0.442	0.659
	C		−1.345	0.179	−0.083	0.934	−0.863	0.388	−0.442	0.659
rs2161961	A	105	2.949	0.003	1.194	0.232	2.639	0.008	2.205	0.027
	G		−2.949	0.003	−1.194	0.232	−2.639	0.008	−2.205	0.027
rs8098539	A	119	1.771	0.077	1.105	0.269	1.302	0.193	1.315	0.189
	G		−1.771	0.077	−1.105	0.269	−1.302	0.193	−1.315	0.189
rs9962277	C	85	1.064	0.287	0.304	0.761	0.732	0.464	0.704	0.481
	T		−1.064	0.287	−0.304	0.761	−0.732	0.464	−0.704	0.481
rs2276134	T	74	0.494	0.622	−0.006	0.995	0.173	0.863	−0.067	0.946
	C		−0.494	0.622	0.006	0.995	−0.173	0.863	0.067	0.946
rs1786581	C	82	−0.110	0.912	0.359	0.720	−0.046	0.963	0.216	0.829
	T		0.110	0.912	−0.359	0.720	0.046	0.963	−0.216	0.829
rs787554	C	101	−0.765	0.444	−1.787	0.074	−0.924	0.355	−2.020	0.043
	T		0.765	0.444	1.787	0.074	0.924	0.355	2.020	0.043
rs787558	T	114	1.612	0.107	0.334	0.738	1.555	0.120	−0.166	0.868
	C		−1.612	0.107	−0.334	0.738	−1.555	0.120	0.166	0.868
rs3892113	T	41	−0.141	0.888	0.257	0.797	−0.219	0.827	0.140	0.889
	G		0.141	0.888	−0.257	0.797	0.219	0.827	−0.140	0.889

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Table 4

TDT analysis of GNAL polymorphisms by the sex of the transmitting parent

SNP	Allele	Paternal transmissions				Maternal transmissions			
		T	NT	χ^2 (1 d.f.)	*P*	T	NT	χ^2 (1 d.f.)	*P*
rs8087266 G/A	G	36	25	1.995	0.158	34	25	1.378	0.240
rs1005270 T/C	T	31	26	0.439	0.508	34	29	0.397	0.529
rs8095592 G/A	G	37	32	0.363	0.547	42	36	0.462	0.497
rs2052661 G/C	G	37	36	0.014	0.907	42	28	2.819	0.093
rs2161961 A/G	A	38	29	1.213	0.271	49	25	7.926	0.005*
rs8098539 A/G	A	43	37	0.450	0.502	46	28	4.423	0.035
rs9962277 C/T	C	27	28	0.018	0.893	33	23	1.795	0.180
rs2276134 T/C	T	23	24	0.021	0.884	24	22	0.087	0.768
rs1786581 C/T	C	28	29	0.018	0.895	34	23	2.136	0.144
rs787554 C/T	C	25	38	2.702	0.100	30	33	0.143	0.705
rs787558 T/C	T	31	33	0.063	0.803	44	30	2.665	0.103
rs3892113 T/G	T	16	10	1.397	0.237	15	18	0.273	0.601

** P* value after 1000 permutations: $P = 0.054$.