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A Functional Variant Provided Further Evidence for the Association of *ARVCF* With Schizophrenia

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Abstract

In a previous linkage disequilibrium mapping study, in the 3′ end of *ARVCF*, we identified one intronic SNP rs165849 and one haplotype block associated with schizophrenia and related disorders. The aim of the present study was to explore whether functional genetic variants in the exonic regions of *ARVCF* included in this haplotype block are responsible for the association observed. To achieve this objective (1) the nine exons included in this haplotype block were resequenced in a group of 242 patients with schizophrenia and related disorders (Case 1). The SNPs identified were genotyped in a hospital-based control group of 373 subjects (Control 1) and an association study was performed. (2) The SNPs showing significant association in this analysis were genotyped in a new group of 102 patients with schizophrenia and related disorders (Case 2) and in a new group of 111 healthy subjects (Control 2). Three dbSNPs (rs35219372, rs5993890, and rs165815) were identified when the nine exons of *ARVCF* were resequenced. rs165815 was associated with schizophrenia and related disorders (homozygote CC OR = 3.39, permutated *P* value = 0.02). When the groups of cases (1 and 2) and controls (1 and 2) were merged, the analysis confirmed the association observed (homozygote CC OR = 3.25 permutated *P* value = 0.02). Given the role of *ARVCF* proposed in the neurodevelopmental hypothesis, our results further support the view that chromosome 22 contains a susceptibility gene, possibly *ARVCF*. The functional variant rs165815, which affects a critical region of ARVCF, is a considerable source of the genetic variability associated with the risk of developing schizophrenia.

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Keywords

schizophrenia; genetic polymorphism; ARVCF; genetic association study

INTRODUCTION

Twin and family studies indicate that genetic factors play a significant role in the transmision and expression of schizophrenia. Genetic association studies in schizophrenia have focused mainly on candidate genes from dopaminergic pathways. Various genes encoding dopamine receptors [*DRD2*; Lafuente et al., 2008] and genes encoding for enzymes involved in dopamine transport and degradation [*MAO-B*, Gassó et al., 2008; *COMT*, Mas et al., 2008; *ACE*, Crescenti et al., 2009] have been implicated in the etiopathology of schizophrenia. Nevertheless, the inconclusiveness of the results suggests that other pathways contribute to the schizophrenia phenotype, for example, via alterations in neural development.

ARVCF (armadillo repeat protein deleted in velo cardio facial syndrome) is one of the most recently identified members of the p120(ctn) subfamily, which plays a key role in the formation of adherens junction complexes [Sirotkin et al., 1997]. More recent work has confirmed that ARVCF is indeed part of the cadherin–catenin complex and may modulate cadherin-mediated junction structures and cell–cell adhesion in various cell types [Kaufmann et al., 2000; Mariner et al., 2000]. It is conceivable that ARVCF influences the migration of neural crest cells by controlling the cell–cell adhesion required for the development of several organs. In turn, the lack of ARVCF might contribute to migratory defect of neural crest cells [Kaufmann et al., 2000]. *ARVCF* was discovered during a search for genes located in the region of chromosome 22q11.2, which is also the location of a relatively common microdeletion alteration, velo cardio facial syndrome (VCFS). Microdeletions, typically about 1.5–3 Mb (megabases) in size and encompassing around 40 genes, result in a wide range of phenotypes. Interestingly, adults with VCFS have high rates of psychosis, most cases fulfilling the diagnostic criteria for schizophrenia, which has an estimated prevalence of 25% [Murphy et al., 1999]. In addition, some studies estimate the frequency of 22q11.2 deletions to be 0.4% in schizophrenia patients [The International Schizophrenia Consortium, 2008]. Thus VCFS has been considered the main risk factor for developing schizophrenia, and the genomic region 22q11.2 one of the most promising candidate regions for association studies. Additional support was provided by genome-wide linkage scans that identified chromosome 22q as one of the loci with the highest likelihood of harboring genes that confer susceptibility to schizophrenia [Badner and Gershon, 2002].

Previous family-based schizophrenia association studies showed that SNPs in the 3′ region of *ARVCF* are linked to the risk of developing schizophrenia [Sanders et al., 2005; Xie et al., 2005], although this association is controversial [Chen et al., 2005; Sanders et al., 2008]. In a previous study, we performed an extensive linkage disequilibrium (LD) analysis, including the 3′ region of *ARVCF* [Mas et al., 2009]. We identified a single marker (rs165849) and a four-marker haplotype block (rs165849-rs2518823-rs887199-rs2239395) associated with this disorder. Although the odds ratio observed was higher in the haplotype analysis than in

the single locus analysis, the former did not increase the statistical significance, thereby indicating that the haplotype effect is attributable to the G allele of rs165849. All the four SNPs studied were intronic and no functional properties were identified through bioinformatic analysis. Here, we expand on our studies in the search for functional SNPs or rare variants in the exonic regions of *ARVCF* included in this block (Fig. 1) and that may be in LD with rs165849 and be responsible for the association observed.

MATERIAL AND METHODS

Study Design

This study was performed following two consecutive stages: (**1**) the nine exons included in the haplotype block of *ARVCF* identified in our previous LD mapping study [Mas et al., 2009] were resequenced in a group of 242 patients with schizophrenia and related disorders (Case 1). The SNPs identified were genotyped in a hospital-based control group of 373 subjects (Control 1) and an association study was performed. (**2**) The SNPs showing significant association in this analysis were genotyped in a new group of 102 patients with schizophrenia and related disorders (Case 2) and in a new group of 111 healthy subjects (Control 2).

Subjects

Case 1—242 subjects diagnosed with schizophrenia and related disorders following DSM-IV criteria [American Psychiatric Association, 1994], consecutively recruited at the Psychiatric Service of the Hospital Clínic (Barcelona, Spain) between 2002 and 2004 who had participated in a previous LD mapping study [Mas et al., 2009], were used to resequence the selected exonic regions of *ARVCF*. Diagnoses were established using the SCID-DSM-IV version [First et al., 1994].

Control 1—A cohort of 373 subjects, who were consecutively recruited at the Trauma Service of the Hospital Clínic and who had also previously participated in the LD mapping study [Mas et al., 2009] as control group, were now genotyped for the SNPs identified in the resequencing analysis. Most of the controls were admitted for hip or knee-joint replacement, pelvic fracture or injury to the upper or lower ribs. A questionnaire eliciting demographic information, data on occupation, smoking habits, and personal medical history was completed in an interview for each patient. Moreover, the clinical information obtained was checked in medical records. Patients were excluded from controls if they reported a history of malignancy or mental disorder.

Case 2—A group of 102 subjects diagnosed with schizophrenia and related disorders were recruited at the same Psychiatric Service of the Hospital Clínic between 2007 and 2008. Diagnoses were established with the same protocols and were performed by the same psychiatric team as Case 1.

Control 2—A group of 111 healthy students of the School of Medicine (University of Barcelona) were recruited during 2009. This group fulfilled the same inclusion/exclusion criteria and completed the same questionnaire described for Control 1. However, in this

control group, the medical information obtained could not be checked in the medical records. This is a limitation of the study as this control group might have included a subject with non-diagnosed or latent schizophrenia.

All participants in the study were Caucasians living in Catalonia. Other ethnic groups were excluded. Catalonia is a region in northeast Spain with 7,210,508 habitants, of whom 96.2% are Caucasians (http://www.idescat.cat/cat/idescat/publicacions/anuari/). Ethnicity was determined by self-reported ancestries; cases and controls reported ancestries for each grandparent. We excluded subjects who mentioned non-European ancestry.

Written informed consent and whole blood samples were obtained from each subject. The study was approved by the Ethics Committee of the Hospital Clínic.

Experimental Procedures

Blood samples were collected from the participants in EDTA (K2EDTA BD Vacutainer EDTA tubes; Becton Dickinson, Franklin Lakes, NJ), and genomic DNA was isolated using DNA purification kits (Puregene; Gentra Systems, Indianapolis, IN). The DNA concentration was determined by means of absorbance (ND1000; Nanodrop, Wilmington, DE).

ARVCF (OMIM 602269) exonic sequences were extracted from Ensembl database (www.ensembl.org/index.html) and confirmed by alignment with genomic sequences (NT_011519.10), which were retrieved from Entrez Gene (www.ncbi.nlm.nih.gov/sites/entrez?db=gene). Primers were designed automatically by means of Oligo 6 software v.6.71 (Molecular Biology Insights, Cascade, CO) and were checked for uniqueness before ordering by means of Blast alignment (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and pre-screened to determine the optimum condition for amplification by means of in silico PCR (http://genome.ucsc.edu/cgi-bin/hgPcr). Primers for amplification and sequencing are shown in Table I. After amplification, a sample of the products was visualized on an agarose gel to confirm the size of the fragment. The remaining amplification product was sent to AGOWA genomics (Berlin, Germany) for purification and sequencing. Sequence analysis was performed using with CodonCode Aligner software (CodonCode Corp., Dedham, MA).

The following three exonic SNPs were identified in the *ARVCF* resequencing procedure performed in Case 1 group: rs35219372, rs5993890, and rs165815. The genotyping of rs35219372 and rs165815 in the rest of the groups was performed by means of allelic discrimination assays TaqMan® (Applied Biosystems, Foster City, CA). To genotype the remaining SNP (rs5993890), we designed a PCR–RFLP method with the primers used to amplify exon 15. The product was digested with Sma I restriction enzyme (New England Biolabs, Ipswich, MA) and visualized on an agarose gel; carriers of the C allele showed two bands of 171 and 256bp, whereas carriers of the T allele showed an undigested fragment of 427bp.

Statistics

Sample size and statistical power calculations were performed with Quanto1.2 software (http://hydra.usc.edu/gxe). Mean and standard deviations were computed for continuous

variables. Univariate analysis (chi-squared test for categorical variables; Student's *t*-test for continuous variables) was used to identify variables associated with the risk of developing schizophrenia. To estimate the independent contribution of each SNP to schizophrenia risk, genotype frequencies were assessed by means of multivariate methods based on logistic regression analysis and analyzed under codominant, dominant, overdominant, recessive, and additive models, adjusting the analysis for these variables (sex, age, and smoking habit) associated with the risk of schizophrenia. The best model was selected using the Akaike information criteria (AIC). For this purpose, we used the SNPassoc R package [González et al., 2007]. Hardy–Weinberg equilibrium and LD relationships between polymorphisms and haplotype block structures were evaluated by Haploview software v.3.2 (http://broad.mit.edu/mpg/haploview). For the haplotype estimations, we used a sliding windows approach with haplo.stats R package. To avoid false-positive results caused by multiple testing, 10,000 permutations were performed to estimate the significance of the best result for both single SNP-based analysis and haplotype-based analysis.

RESULTS

The demographic characteristics of the overall group of cases and controls are summarized in Table II. Patients with schizophrenia and related disorders included in Cases 1 and 2 did not differ significantly in terms of either sociodemographic or diagnosis features. Differences in the proportion of males, age, and smoking habit observed on the comparison of the overall groups of cases and controls are also observed in the comparison of Case 1 and Control 1 groups (stage 1), and in the comparison of Case 2 and Control 2 (stage 2). Because of these differences, the statistical analysis was adjusted to account for these features.

In stage (1), the nine *ARVCF* exons encompassing the haplotype block formed by rs165849, rs2518823, rs887199, and rs2239395 were resequenced in the group of patients with schizophrenia and related disorder (Case 1). Three dbSNPs were identified (Table III), including two synonymous SNPs (rs35219372 and rs5993890) in exon 20 and one non-synonymous SNP (rs165815) in exon 19 (Fig. 1). These SNPs were genotyped in the hospital-based control group (Control 1) and association analysis showed that rs165815 was associated with schizophrenia (permutated *P* value = 0.007; statistical power 87%) (Table III). The genotype frequencies of this SNP analyzed under codominant, dominant, overdominant, recessive, and additive models are shown in Table IV. According to the AIC, the best model to explain the association is the additive model. As shown by this model and the dominant model, carriers of the C allele of rs165815 (homozygotes and heterozygotes) had almost a double risk of developing schizophrenia and related disorders (OR = 1.6, CI = 1.1–2.4), whereas homozygotes for this allele had more than three times the risk of developing the disorder (OR = 3.3, CI = 1.1–10.1), as shown by the codominant model. When we stratified this analysis by diagnoses and analyzed the schizophrenia strata, very similar results were obtained (data not shown).

In order to confirm the association of rs165815 with schizophrenia and related disorders, we genotyped it in a new group of cases recruited in the same hospital (Case 2) and in a new group of healthy subjects from the same geographical area (Control 2). The association

analysis reported similar results with both allele frequencies (OR = 1.34, CI = 0.7–2.1) and genotype frequencies (OR = 2.8, CI = 0.6–14.9) (data not shown). However, the associations did not achieve significant results, probably because of the small sample size. When the groups of cases and controls were pooled (Table II), the analysis of SNP-based allelic associations (allele frequencies 0.187 in cases and 0.132 in controls; OR = 1.53; CI = 1.1–2.0; $P = 0.003$; permutated P value = 0.007; statistical power 96%) and the genotype frequencies (Table V) confirmed the association observed in stage (1).

The four SNPs analyzed in our previous LD mapping study and the three SNPs identified in the resequencing of exonic sequences of *ARVCF* formed a haplotype block identified by searching for the solid spine of strong LD (Fig. 1). One of the haplotypes of this block (haplotype 5), with the risk alleles associated with schizophrenia for rs165849 and rs165815, was associated with the risk of schizophrenia and related disorders ($P = 0.01$). However, it did not survive the correction for multiple testing. When we constructed a diplotype considering the single marker identified in our previous study (rs165849) and the SNP associated with schizophrenia in the present work (rs165815), significant associations were obtained. The diplotype $rs165849^{G}$-$rs165815^{C}$, with the two alleles associated with schizophrenia, was more common in cases (0.19) than in controls (0.13) (OR = 1.4, CI = 1.1–2.1; P value = 0.006; permutated P value = 0.01).

DISCUSSION

In a previous LD mapping study [Mas et al., 2009], in the 3′ end of *ARVCF* we identified one intronic SNP rs165849 and one haplotype block associated with schizophrenia and related disorders. The aim of the present study was to explore whether functional genetic variants in the exonic regions of *ARVCF* included in this haplotype block are in LD with rs165849 and whether they are responsible for the association observed. To achieve this objective, we resequenced the exons embedded in this haplotype block.

We identified three dbSNPs in this region (rs35219372, rs5993890, and rs165815) and one single marker association between rs165815 and schizophrenia and related disorders. Moreover, the haplotype analysis revealed that this non-synonymous SNP is in high LD with the previous marker rs165849. This is not the first time that these SNPs have been associated with schizophrenia. A haplotype block spanning the *COMT* gene and the 3′ portion of *ARVCF* were linked with schizophrenia as reported by Sanders et al. [2005] in families with schizophrenia. This haplotype block includes rs165849. In Chinese family trios, Xie et al. [2005] found a significant association between rs165815 and schizophrenia. However, these results were not replicated in other studies [Chen et al., 2005; Sanders et al., 2008]. Ambiguous findings of association studies between genetic polymorphisms and schizophrenia are frequent. There are no biological markers that make the disorder syndromic, and diagnosis is based on a combination of clinical observations. Schizophrenia, as defined by current diagnostic criteria, may also include a number of heterogeneous diseases [Owen et al., 2004]. Phenotypic heterogeneity is a well-known limitation in genetic studies in psychiatry and may be responsible for discrepancies between published results or for lack of replication in independent samples. For this reason, we enlarged our initial group of cases with new patients recruited in the same Psychiatry Service and diagnosed by the

same psychiatrist to ensure greater phenotypic homogeneity and to guard against confounding population stratification. The association of rs165815 with schizophrenia and related disorders observed in our initial group of cases (Case 1) and controls (Control 1) was confirmed when these groups were extended with two new groups (Case 2 and Control 2). A similar magnitude of the effect and significance was obtained in the same direction with the same SNP. This confirmation rejected the probability of type-I error and increased the confidence in the finding.

The associated SNP is a missense mutation localized in exon 15 which produces an amino acid change, arginine for glutamine, in the protein position 906 (this position may differ depending on the splice variant). This position corresponds to the C-terminus region of ARVCF. This region is required for the correct translocation of the protein to the nucleus, through a nuclear localization signal[Kaufmann et al., 2000] and for selective protein–protein interaction [Waibler et al., 2001]. The cellular distribution of ARVCF is determined not only by the presence or the absence of an appropriate interaction partner but also by the cellular context [Waibler et al., 2001]. *ARVCF* is expressed in multiple regions of the early development, adolescent, and mature mouse brain [Maynard et al., 2003] and is also expressed in human brain, including fetal [Sirotkin et al., 1997]. Members of the human catenin family play key roles in developmental patterning [Krubitzer and Kahn, 2003; McCrea and Park, 2007]. Hence, *ARVCF* may be a candidate gene for susceptibility to schizophrenia via alterations in neural development, which is linked with the neurodevelopmental hypothesis of schizophrenia [Weinberger, 1987].

ARVCF is found in the region of chromosome 22q11.2, one of the most promising candidate regions for association studies in schizophrenia because of its involvement in VCFS and genome-wide linkage scans that identify chromosome 22q as one of the loci with the highest likelihood of harboring schizophrenia risk genes [Badner and Gershon, 2002]. *COMT* is another gene found close to *ARVCF* in this region and is probably one of the genes with a better a priori case for involvement in schizophrenia. Given its localization and its function in monoamine metabolism, together with the existence of a functional polymorphism (Val158Met or rs4680), *COMT* has become a common target for genetic research, with more than 15 published case–control studies. As would be expected for a putative risk allele of small effect, numerous studies include both positive and negative findings [Williams et al., 2007]. The discrepancy of results is potentially consistent with true association where replication is hampered by lack of power. However, three recent meta-analyses of the published case–control literature found no significant effect of the Val/Met locus [Fan et al., 2002; Glatt et al., 2003; Munafo et al., 2005]. The soundest results in association with schizophrenia risk are based on haplotype blocks including SNPs of the 3′ region of *COMT* [Shifman et al., 2002]. Moreover, some of the family studies analyzing *ARVCF* considered haplotype blocks spanning *COMT* and *ARVCF*, including the functional polymorphisms *COMT* Val158Met [Li et al., 2000; Sanders et al., 2005].

Additional studies that replicate the observed association in populations of distinct ancestry are required to determine whether this variant allele at ARVCF is a universal risk factor or whether it is restricted to the Spanish population. Moreover, functional analysis of the impact of rs165815 on the activity of ARVCF is also required.

Given the role of *ARVCF* proposed in the neurodevelopmental hypothesis [Chen et al., 2005], our results add further support to the view that chromosome 22 contains a susceptibility gene, possibly *ARVCF*, which is in LD with *COMT* alleles. The functional variant rs165815, which affects a critical region of ARVCF, is an important source of the genetic variability associated with the risk of developing schizophrenia.

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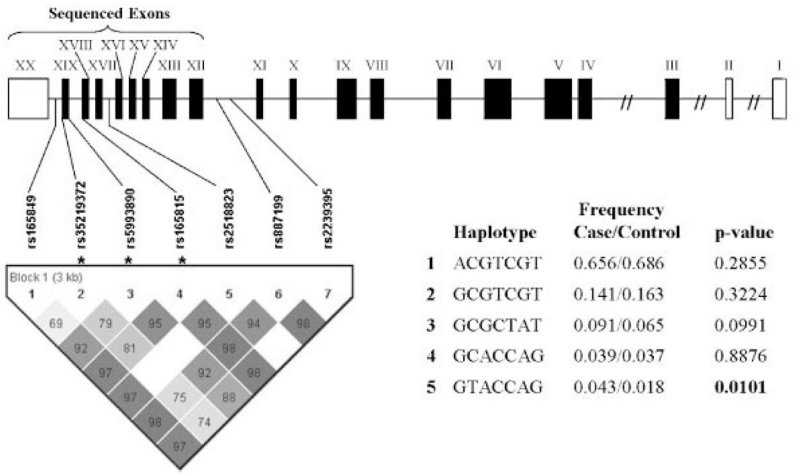

FIG. 1.

Linkage disequilibrium pattern in *ARVCF*. Standard color scheme in Haploview is used to display logarithm of odds (LOD) and the D′. Estimated statistics of D′ is shown in each box (are not labeled if D′ = 1). The *ARVCF* gene structure is illustrated at the top (white boxes are untranscribed exons and black boxes are coding exons). The relative position of the SNPs forming the four-marker haplotype block associated with schizophrenia in our previous study and those identified in the former (marked with an asterisk) are also shown. The left inset shows the seven marker haplotype frequencies in cases and controls and the *P* values for the association analysis.

TABLE I

Sequence of the Primers Used to Amplify the Nine Exons Included in the *ARVCF* Haplotype Block Associated With Schizophrenia

***ARVCF* exon**	**Primer name**	**Primer sequence 5′ → 3′**
Exon 12	U-EXO9	CCA CTG CCA CCA ACT CCT ACC
	L-EXO9	TCA CTA CCA GGG CAA TCC
	SEQ-EXO9	TGC CCA AAC TCT GAC AAC GGT
Exon 13	U-EXO10	ATG CGG GTC TTG TTG CTA GGA
	L-EXO10	TTG CCC GCA GCC CAT ACA C
	SEQ-EXO10	GGA CTT GCC CAC CCT GCC CGA
Exon 14	U-EXO11	GTG TAT GGG CTG CGG GCA ATG
	L-EXO11	TCT TTG TTG CGC CGG TCC AG
	SEQ-EXO11	TAT GGG CTG CGG GCA ATG CGT
Exon 15, 16, and 17	U-EXO12/13/14	GCC TGG GCA CCG AAC TGT
	L-EXO12/13/14	TGG CAG CGT GCT GTC ATC GAA
	SEQ-EXO12	CAC ACC TGG AGG CCA CGA GAG
	SEQ-EXO13/14	CGT GGT GGC GGT GCT CAA CAC
Exon 18	U-EXO15	GAG GGG AGT CTG GAG AGT TGG
	L-EXO15	CAC AGA CTG ACT GGC GTG AAG
	SEQ-EXO15	GAC CTG CCC ACA GCC ACA TGA
Exon 19	U-EXO16	TCC CTG CGC CCT TGG TGC TAC
	L-EXO16	TGC CTG GTC TTT CCG GGA ATG
	SEQ-EXO16	AAC AAG AAG CCC TGG CCC AGA
Exon 20	U-EXO17	GGC CAC CCT GAG CAG ATC GTG
	L-EXO17	TGA CCT TCG GCA GTG GCT GGG
	SEQ-EXO17	AGC AGA TCG TGC CGT GGA GC

TABLE II

Characteristics of the Overall Group of Cases (Cases 1 and 2) and the Overall Group of Controls (Controls 1 and 2) for the Schizophrenia Risk Study

	Overall controls (Controls 1 + 2)	Overall cases (Cases 1 + 2)
N	484	344
Age	45.02 ± 21	32.05 ± 12[b]
Sex (men)	261/484 (53.9%)	198/344 (57.5%)[b]
Smokers[a]	206/484 (42.5%)	126/230 (54.7%)[b]
Diagnosis		
Schizophrenia		226 (65.7%)
Schizoaffective disorder		36 (10.4%)
Acute psychotic disorder		68 (19.8%)
Delusional (paranoid) disorder		11 (3.2%)
Schizotypal disorder		3 (0.8%)

[a] Smoker group includes ex-smokers. For some patients, no information on tobacco use was available (114 cases missing).

[b] $P < 0.001$ (cases vs. controls).

TABLE III

ARVCF SNPs Detected After Resequencing and Analysis of Allele Frequencies and Association Performed in Stage (1)

SNP	Chr position	Alleles[a]	Case 1	Control 1	OR	95% CI	*P* value[b,c]
rs35219372	18338811	C/T	0.049	0.030	1.66	0.9–3.0	0.0951 (n.s.)
rs5993890	18338829	G/A	0.091	0.062	1.53	0.9–2.3	0.0551 (n.s.)
rs165815	18339473	T/C	0.194	0.128	1.63	1.2–2.2	0.0024 (**0.007**)

OR, odds ratio; CI, 95% confidence interval; n.s., not significant.

[a] Underlined allele is the associated allele.

[b] *P*-value adjusted covariation by sex, age, and smoking habit.

[c] In parenthesis *P*-value obtained in the permutation analysis.

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TABLE IV

Genotype Analysis of rs165815 Under Codominant, Dominant, Overdominant, Recessive, and Additive Models in Stage (1)

	Control 1; n (%)	Case 1; n (%)	OR	95% CI	*P* value[a,b]	AIC[c]
Codominant						
T/T	261 (75.9)	154 (65.5)	1.00		0.009 (**0.02**)	778.7
C/T	78 (22.7)	71 (30.2)	1.54	1.06–2.25		
C/C	5 (1.5)	10 (4.3)	3.39	1.14–10.10		
Dominant						
T/T	261 (75.9)	154 (65.5)	1.00		0.006 (**0.01**)	778.7
C/T-C/C	83 (24.1)	81 (34.5)	1.65	1.15–2.38		
Recessive						
T/T-C/T	339 (98.5)	225 (95.7)	1.00		0.039 (n.s.)	781.8
C/C	5 (1.5)	10 (4.3)	3.01	1.02–8.93		
Overdominant						
T/T-C/C	266 (77.3)	164 (69.8)	1.00		0.042 (n.s.)	781.9
C/T	78 (22.7)	71 (30.2)	1.48	1.01–2.15		
Additive	344 (59.4)	235 (40.6)	1.63	1.18–2.25	0.002 (**0.007**)	**777.0**

OR, odds ratio; CI, 95% confidence interval; n.s., not significant; AIC, Akaike Information Criterion.

[a] *P*-value adjusted covariation by sex, age, and smoking habit.

[b] In parenthesis *P*-value obtained in the permutation analysis.

[c] AIC attempts to find the minimal model that correctly explain the data.

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TABLE V

Genotype Analysis of rs165815 Under Codominant, Dominant, Overdominant, Recessive, and Additive Models in Stage (2)

	Overall controls; n (%)	Overall cases; n (%)	OR	95% CI	*P* value[a,b]	AIC[c]
Codominant						
T/T	341 (74.9)	225 (67.0)	1.00		0.008 (**0.02**)	1,075
C/T	107 (23.5)	96 (28.6)	1.36	0.98–1.88		
C/C	7 (1.5)	15 (4.5)	3.25	1.30–8.09		
Dominant						
T/T	341 (74.9)	225 (67.0)	1.00		0.014 (**0.04**)	1,077
C/T-C/C	114 (25.1)	111 (33.0)	1.48	1.08–2.01		
Recessive						
T/T-C/T	448 (98.5)	321 (95.5)	1.00		0.013 (**0.04**)	1,076
C/C	7 (1.5)	15 (4.5)	2.99	1.21–7.42		
Overdominant						
T/T-C/C	348 (76.5)	240 (71.4)	1.00		0.108 (n.s.)	1,080
C/T	107 (23.5)	96 (28.6)	1.30	0.94–1.79		
Additive	455 (57.5)	336 (42.5)	1.53	1.14–1.99	0.003 (**0.007**)	**1,074**

OR, odds ratio; CI, 95% confidence interval; n.s., not significant; AIC, Akaike Information Criterion.

[a] *P*-value adjusted covariation by sex, age, and smoking habit.

[b] In parenthesis *P*-value obtained in the permutation analysis.

[c] AIC attempts to find the minimal model that correctly explain the data.

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