Original Paper

Human Heredity

Hum Hered 2009;67:163–173
DOI: 10.1159/000181154

Received: May 19, 2008
Accepted: May 20, 2008
Published online: December 15, 2008

Evidence for a Role of the *NOS1AP (CAPON)* Gene in Schizophrenia and Its Clinical Dimensions: An Association Study in a South American Population Isolate

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Key Words
NOS1AP · Schizophrenia · Clinical heterogeneity · Genetic association · Psychiatric genetics

Abstract
Background/Aims: Recent studies have implicated a region on chromosome 1q21-23, including the *NOS1AP* gene, in susceptibility to schizophrenia. However, replication studies have been inconsistent, a fact that could partly relate to the marked psychopathological heterogeneity of schizophrenia. The aim of this study is to evaluate association of polymorphisms in the *NOS1AP* gene region to schizophrenia, in patients from a South American population isolate, and to assess if these variants are associated with specific clinical dimensions of the disorder. ***Methods:*** We genotyped 24 densely spaced SNPs in the *NOS1AP* gene region in a schizophrenia trio sample. The transmission disequilibrium test (TDT) was applied to single marker and haplotype data. Association to clinical dimensions (identified by factor analysis) was evaluated using a quantitative transmission disequilibrium test (QTDT). ***Results:*** We found significant association between eight SNPs in the *NOS1AP* gene region to schizophrenia (minimum p value = 0.004). The QTDT analysis of clinical dimensions revealed an association to a dimension consisting mainly of negative symptoms (minimum p value 0.001). ***Conclusions:*** Our findings are consistent with a role for *NOS1AP* in susceptibility to schizophrenia, especially for the 'negative syndrome' of the disorder.

Introduction

Schizophrenia is a severe and debilitating psychiatric disorder which is among the leading causes of morbidity and long-term disability world-wide [1]. Family, twin, and adoption studies indicate that schizophrenia has an important genetic component [2], and linkage studies

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have implicated a number of genomic regions potentially involved in disease susceptibility [3–9]. A region of 12 cM on chromosome 1q21-22 produced a parametric multipoint LOD score of 6.5 in a sample of 22 Canadian families [5]. This region harbours several candidate genes for schizophrenia, including the Regulator of G Protein Signalling 4 (*RGS4*), the Nitric Oxide Synthase 1 (neuronal) Adaptor Protein (*NOS1AP*; also called *CAPON*), and the U2AF Homology Motif Kinase 1 (*UHMK1*). The *NOS1AP* gene is of particular interest due to its involvement in the glutamatergic neurotransmission system, a pathway thought to play a role in the aetiology of schizophrenia [10]. Several studies have shown significant association between markers in the *NOS1AP* gene and schizophrenia, in samples from Canada ($p = 0.0016$), and China ($p = 0.000017$) [11, 12]. Also, we recently analysed marker D1S1679, located 23.5 kb downstream of the *NOS1AP* gene, in a schizophrenia trio sample from a South American population isolate (Antioquia) [13–15] and found a significant association ($p = 0.019$) [16]. However, other studies have failed to detect such an association [17]. The discordance of the *NOS1AP*-schizophrenia association results could relate to the marked psychopathological heterogeneity of schizophrenia. Patients can differ widely with respect to age of onset, inter-episode recovery and prominence of both positive and negative symptoms [18]. Since this clinical variability could reflect an underlying genetic heterogeneity, it seems interesting to explore the analysis of clinical dimensions – quantifiable symptom complexes varying across affected individuals – in studies aimed at the identification of genetic loci predisposing to schizophrenia [19, 20].

To follow-up our previous report implicating marker D1S1679, we obtained data for SNPs in the *NOS1AP* gene typed in an expanded collection of Antioquian schizophrenia trios. We performed single-marker as well as haplotype-based TDT analyses. In view of the psychopathological heterogeneity of schizophrenia, we also used a dimensional approach to evaluate association between clinical features and the *NOS1AP* gene. These analyses confirmed association of *NOS1AP* polymorphisms both to disease and to a clinical dimension comprising negative symptoms of schizophrenia.

Methods and Materials

Study Sample and Clinical Procedures

The study sample consisted of 102 patients diagnosed with schizophrenia (79 males, 23 females; mean age 34.3 ± 10.2 years; mean age of onset 21 ± 7.2 years; mean duration of the illness 13.4 ± 8.7 years) and their nuclear families. For 38 of the patients, both parents were available (complete trios); for 45, one parent and one full sibling were available (parent-sibling-trios), for 3 patients, one parent and two full sibs were available, and for 13 patients, only one parent could be obtained (duos). Three families were of a different structure, two of them comprising the index case, a sibling and a paternal uncle resp. aunt, one consisting of the index case and two full siblings.

Patients with a diagnosis of schizophrenia were recruited in the municipalities of Medellín and Envigado (Antioquia, Colombia) at Hospital Mental de Antioquia, Hospital San Vicente de Paúl, Clínica Sameín, Clínica Insam, and the Mental Health Centre of Envigado.

Patients were assessed by a psychiatrist (J.G., C.L., M.R.) using the Spanish version of the Diagnostic Interview for Genetic Studies (DIGS) previously validated in Colombia [21, 22]. All patients were in remission during the entire assessment procedure. A final diagnosis using DSM-IV-TR criteria [23] was reached through a best estimate procedure which required two experienced psychiatrists (other than the one conducting the interview; C.P., G.R.) to independently reach the same diagnosis of schizophrenia based on the DIGS and clinical records. If no consensus diagnosis could be obtained, a third psychiatrist (J.O.) was consulted. If still no consensus could be reached, the subject was excluded from the study. Additionally, inclusion criteria required at least six out of the eight great-grandparents to be of Antioquian origin. Patients with mental retardation and/or neurological lesions were excluded from the study.

The clinical dimensions were obtained from the Scales of Assessment of Positive and Negative Symptoms (SAPS and SANS) [24–26] by factor analysis. The SANS and SAPS were applied to all schizophrenic patients by an experienced psychiatrist. To comply with the minimum sample size recommended for factor analysis [27, 28], the scales were also applied to an additional sample of 150 schizophrenic patients diagnosed according to the same diagnostic criteria and with similar clinical parameters, making for a total sample of 252. However, these additional individuals were not available for genotyping.

This study was approved by the Bioethics Committee of the Universidad de Antioquia (Colombia) and by the Ethics Committees of all participating institutions.

Marker Selection and Genotyping

We selected 24 SNP markers that together cover 314 kb across the *NOS1AP* gene region. A schematic overview of the markers chosen and their location with respect to *NOS1AP* is given in figure 1. The 24 markers included 9 SNPs from the original association study of *NOS1AP* [11] (numbers in brackets refer to SNP numbering as in fig. 1): rs1572495 [#3], rs1538018 [#4], rs945713 [#5], rs1415263 [#7], rs3924139 [#8], rs4145621 [#11], rs2661818 [#16], rs3751284 [#17], and rs348624 [#22], and additional SNPs selected from evolutionary conserved regions within or close to *NOS1AP* [29]: rs12090585 [#1], rs11579080 [#2], rs6664602 [#6], rs4592244 [#9], rs4657179 [#10], rs4656362 [#12], rs6680461 [#13], rs4657181 [#14], rs10800405 [#15], rs1504430 [#18], rs17468951 [#19], rs12122048 [#20], rs905720 [#21], rs1123005 [#23], rs11806859 [#24).

Genomic DNA was extracted from blood samples using standard laboratory procedures.

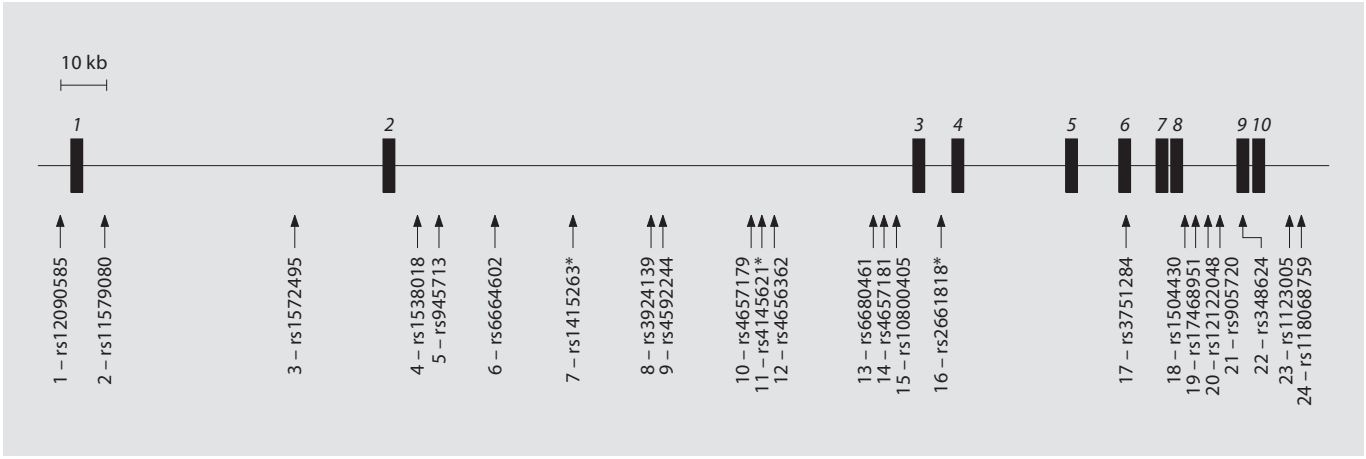


Fig. 1. Location of markers genotyped in the *NOS1AP* gene region (markers rs6664602 [#6] and rs4656362 [#12] were excluded from subsequent analyses; see text). The locations of *NOS1AP* exons are indicated by black boxes; exon numbers are in italics. Consecutive marker numbers from 1 to 24 are included for easier comparison with the LD plot in figure 2a. Markers significantly associated with schizophrenia in Brzustowicz et al. [11] are indicated with an asterisk.

A multiplex PCR assay was developed to genotype multiple SNPs. The multiplex PCR design was based on software described in [30] and genotyping was conducted via a ligation detection assay and scored on a Luminex 100 [31, 32]. Primer sequences and amplification conditions can be obtained from the authors.

Data Analysis

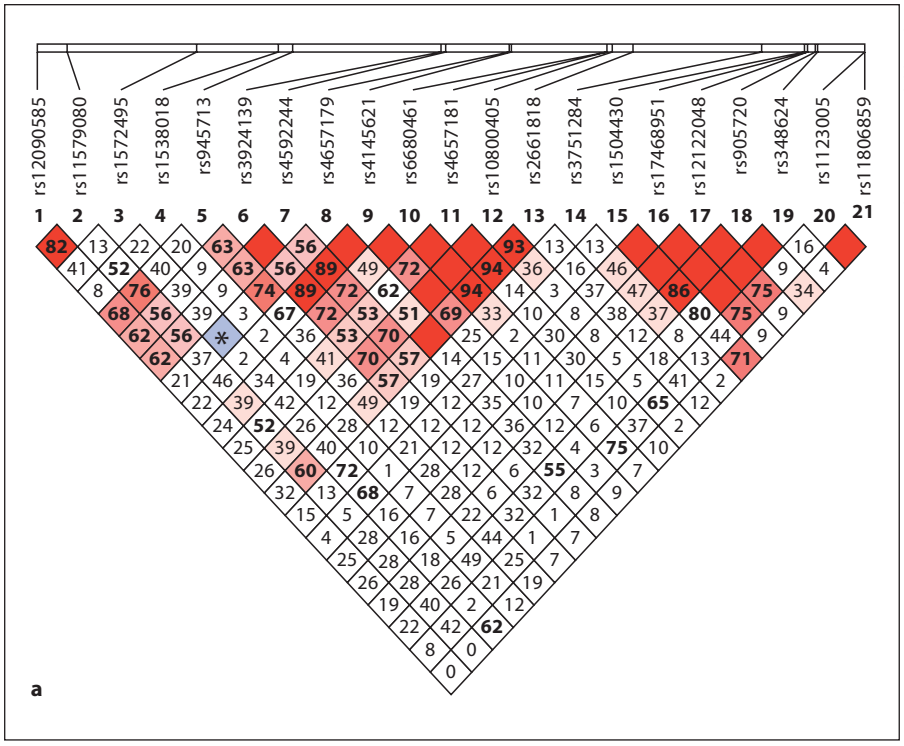
Hardy-Weinberg equilibrium was evaluated separately in founders and cases using the GENEPOP program [33] (available online at http://wbiomed.curtin.edu.au/genepop/). Allele frequencies of non-transmitted alleles were estimated using TDTPHASE v.2.4 [34].

The Transmission Disequilibrium Test (TDT) [35] for single markers was carried out as implemented in the TRANSMIT program, version 2.5.4 [36]. TRANSMIT carries out a generalised transmission/disequilibrium test that can be applied to situations of uncertain allele or haplotype transmission, allowing the analysis of data from patients with only one available parent, and to multilocus data even under uncertain phase, thereby permitting the use of all available genotypic data, including all nuclear family structures (data from unaffected siblings are used to infer parental genotypes and haplotypes). The statistic estimated by TRANSMIT follows a χ^2 distribution and is calculated as $(O - E)^2 = Var(O - E)$, where O and E refer to the number of observed and expected transmissions of alleles/haplotypes. As an alternative method of determining the p value, the program employs a bootstrap method which provides more accurate results than the χ^2 approximation for small samples.

Linkage disequilibrium across the *NOS1AP* gene was evaluated using Haploview v3.2 [37] based on parental genotypes. To compare the LD structure in our samples to the LD structure in the European population to which the Antioquian population is very close with 80% autosomal European ancestry [13], we downloaded the HapMap data for Europeans for the same region from the HapMap project website (http://www.hapmap.org) and displayed the LD plot using Haploview. Regions with the highest levels of linkage disequilibrium were used for haplotype-based TDT with WHAP v.2.09 [38]. For these analyses, the prevalence of the disease in the population was set to 0.5% [1], and the minimum frequency for a haplotype to be included in the analysis was fixed at 1%. As WHAP only accepts data from parent-offspring trios or duos and cannot accommodate other relatives, WHAP analyses included only 99 index cases: 38 complete trios and 61 duos. Two tests were performed: an omnibus haplotype test (testing the effects of each haplotype against a reference haplotype) and a haplotype-specific test (testing the effect of each haplotype against all others).

Clinical dimensions were obtained by principal component factor analysis performed on the individual items of the SANS and SAPS. Sampling adequacy was evaluated with the Kaiser-Meyer-Olkin measure (KMO) [39]. The resulting KMO of 0.89 indicated good variable factorability (i.e., the variables under study have a low partial correlation coefficient). Factor analysis was then performed using SPSS 13.0, using the Scree criterion for factor selection [40]. In order to assign items to factors, only the items with a loading of 0.40 or greater were taken into account. The factor solution was then rotated (using the VARIMAX procedure) and factor scores calculated using regression. All factors have eigenvalues >1.0, indicating that they account for more variance than any single SANS or SAPS item. Four of the index cases had no quantitative data available, thus reducing the sample size for these analyses to 98.

The factor scores were normalised before QTDT analysis. First, all 98 individuals were ranked based on their dimensional score. Ranks were converted to percentiles [rank/(N + 1), where N is the number of individuals] and z-scores obtained using the inverse standard normal cumulative distribution. The z-scores were then used as input for the quantitative TDT analysis with the QTDT program v.2.5.1 [41].



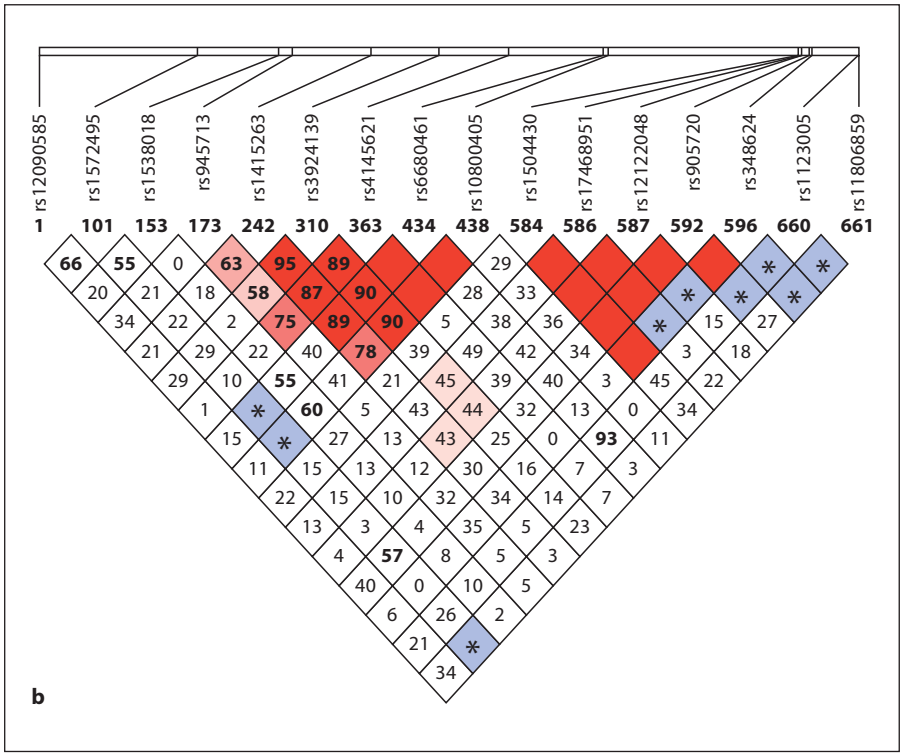


Fig. 2. a LD structure between 22 genotyped SNPs across the *NOS1AP* gene in the Antioquian sample. Marker numbers (1 to 24) are as in figure 1. Values in the boxes refer to % D′. Where no number is shown, D′ = 1.0. Red and pink (black and grey in printed version) boxes indicate a LOD score of ≥2.0 (D′ significant); blue (marked by asterisk) and white boxes indicate a LOD score of <2.0 (D′ not significant). **b** LD structure across the *NOS1AP* gene based on data for the European HapMap population (CEU; http://www.hapmap.org). Only HapMap SNPs also genotyped in this study are shown. Since not all SNPs included here are HapMap SNPs, the set of SNPs displayed for the European population is smaller than the one typed in the Antioquian population.

Table 1. Single marker TDT (TRANSMIT) for 22 SNPs within the CAPON gene

SNP	Number[a]	allele	allele frequency[b]	O	E	Var(O – E)	χ^2 (1 d.f.)	p value	bootstrap p value[c]
rs12090585	1	G	0.667	132	132.98	19.558	0.0487	0.83	0.82
		A	0.333	68	67.024				
rs11579080	2	G	0.623	110	109.87	13.812	0.0012	0.97	0.97
		T	0.377	64	64.129				
rs1572495	3	G	0.907	180	182.74	8.5861	0.8728	0.35	0.40
		A	0.093	24	21.263				
rs1538018	4	G	0.650	146	142.31	18.396	0.7392	0.39	0.39
		C	0.350	60	63.688				
rs945713	5	G	0.470	120	109.93	18.947	***5.3500***	***0.021***	***0.011***
		A	0.530	82	92.068				
rs1415263	7	C	0.478	87	97.35	19.523	***5.4866***	***0.019***	***0.015***
		T	0.522	117	106.65				
rs3924139	8	C	0.426	95	105.48	18.506	***5.9341***	***0.015***	***0.007***
		T	0.574	107	96.521				
rs4592244	9	G	0.426	95	105.48	18.506	*5.9341*	*0.015*	*0.007*
		A	0.574	107	96.521				
rs4657179	10	G	0.802	164	165.42	13.308	0.1518	0.70	0.70
		T	0.198	40	38.579				
rs4145621	11	C	0.570	138	126.19	18.856	***7.3991***	***0.007***	***0.004***
		T	0.430	64	75.812				
rs6680461	13	T	0.740	136	144.44	15.679	***4.5391***	***0.033***	***0.028***
		G	0.260	68	59.564				
rs4657181	14	A	0.567	143	131.54	19.618	***6.6972***	***0.010***	***0.006***
		T	0.433	63	74.462				
rs10800405	15	C	0.724	132	139.15	15.149	3.3715	0.07	0.05
		G	0.276	66	58.853				
rs2661818	16	C	0.651	128	124.71	16.631	0.6498	0.42	0.39
		G	0.349	62	65.287				
rs3751284	17	C	0.638	105	114.27	20.327	***4.2276***	***0.040***	***0.019***
		T	0.362	95	85.73				
rs1504430	18	C	0.681	129	134.41	19.001	1.5430	0.21	0.25
		T	0.319	75	69.585				
rs17468951	19	A	0.639	139	136.23	18.138	0.4228	0.52	0.54
		G	0.361	65	67.769				
rs12122048	20	G	0.633	138	135.1	17.474	0.4817	0.49	0.47
		A	0.367	64	66.901				
rs905720	21	C	0.696	143	139.31	16.922	0.8057	0.37	0.39
		T	0.304	59	62.692				
rs348624	22	C	0.845	171	171.12	11.231	0.0013	0.97	0.98
		T	0.155	33	32.877				
rs1123005	23	A	0.854	169	173.3	11.254	1.6394	0.20	0.28
		A	0.718	55	56.138				
rs11806859	24	G	0.282	147	145.86	15.976	0.0810	0.78	0.80
		A	0.718	55	56.138				

Nominally significant χ^2 and p values are italicized and bold. Markers belonging to LD regions 1 (top) and 2 (bottom) are shaded.
O = observed transmissions; E = expected transmissions; d.f. = degree of freedom.
[a] SNP number as in figure 1; [b] in untransmitted chromosomes; [c] based on 1000 bootstrap samples.

Table 2. Results of the transmission disequilibrium test of the haplotype containing the ten SNPs forming LD region 1

Haplotype #	rs945713 [#5]	rs1415263 [#7]	rs3924139 [#8]	rs4592244 [#9]	rs4657179 [#10]	rs4145621 [#11]	rs6680461 [#13]	rs4657181 [#14]	rs10800405 [#15]	rs2661818 [#16]	Frequency	Omnibus test (OT) overall p = 0.348			Haplotype-specific test (HS)	
												OR_{OT}	lower limit[a]	upper limit[a]	OR_{HS}	p value
1	A	C	T	A	G	T	T	T	C	G	0.281	reference haplotype			0.54	***0.04***
2	G	T	C	G	T	C	G	A	G	C	0.131	1.79	0.73	4.37	1.13	0.75
3	G	T	C	G	G	C	G	A	G	C	0.096	2.29	0.85	6.18	1.48	0.38
4	G	T	C	G	G	C	T	A	C	C	0.084	3.37	1.21	9.40	2.24	0.09
5	G	T	C	G	G	C	T	T	C	G	0.059	3.40	1.07	10.82	2.22	0.14
6	A	T	C	G	G	C	G	A	G	C	0.056	1.23	0.36	4.24	0.77	0.65
7	A	C	T	A	G	C	T	A	C	C	0.053	1.67	0.43	6.42	0.99	0.99
8	A	C	T	A	G	T	T	A	C	C	0.048	0.63	0.16	2.40	0.39	0.15
9	G	C	T	A	G	C	T	A	C	C	0.03	1.33	0.25	6.98	0.87	0.86
10	G	T	C	G	T	C	T	A	C	C	0.03	1.12	0.21	5.93	0.72	0.68
11	A	T	C	G	G	C	T	A	C	C	0.028	4.86	1.06	22.24	3.03	0.14
12	G	C	T	A	G	T	T	T	C	G	0.027	1.55	0.25	9.78	0.88	0.88
13	G	T	T	A	G	C	T	A	C	C	0.025	4.19	0.84	20.80	2.63	0.23
14	G	T	T	A	T	C	T	A	C	C	0.015	3.30	0.40	27.33	2.00	0.51
15	A	C	T	A	T	C	G	A	G	C	0.014	0.95	0.06	14.07	0.56	0.66
16	G	T	C	G	T	C	T	T	C	C	0.013	0.92	0.08	10.21	0.59	0.65
17	G	C	T	A	G	C	G	A	G	C	0.011	0.28	0.03	2.77	0.19	0.15

OT = Omnibus test; HS = haplotype-specific test; OR = odds ratio.

[a] Upper and lower limit of the 95% confidence interval for the OR of the omnibus test. WHAP does not calculate the 95% confidence intervals for the ORs of the haplotype-specific test; instead, a p value is given for each of the tested haplotypes.

Nominally significant p values are printed in bold italics.

Results

All markers tested were found to be in Hardy-Weinberg equilibrium, with exception of marker rs4656362 [#12] (p = 0.0197). Marker rs6664602 [#6] was found not to be polymorphic in the Antioquian population. These two markers were therefore excluded from all further analyses.

LD Analysis

The pattern of LD between the SNPs genotyped in the Antioquian sample, as determined by Haploview, is represented in figure 2a. Two regions of strong LD are apparent, the first one covering markers rs945713 [#5] to rs2661818 [#16], spanning 129.1 kb and comprising introns 2 and 3 of *NOS1AP* (region 1), the second one covering markers rs1504430 [#18] to rs348624 [#22] and spanning 5.1 kb mostly within intron 8 (region 2).

For comparison, the LD structure of the European HapMap population (CEU) for a subset of the markers typed in this study is displayed in figure 2b. It shows great similarity to the pattern seen in the Antioquian sample, with two regions of high LD in the same locations as observed in Antioquia.

Single Marker Association Tests

The results of the single marker TDT are summarised in table 1. Of the 22 SNPs examined, eight showed significant association to schizophrenia (overtransmitted alleles are shown in parentheses): rs945713 [#5] (G), rs1415263 [#7] (T), rs3924139 [#8] (T), rs4592244 [#9] (A), rs4145621 [#11] (C), rs6680461 [#13] (G), rs4657181 [#14] (A), and rs3751284 [#17] (T). For all eight markers, both the p value based on the χ^2 approximation and the bootstrap p value are nominally significant. Of these eight markers, seven are in high LD with each other and are located in LD region 1, while the remaining marker, rs3751284 [#17], is not in strong LD with any other marker (fig. 2a).

Table 3. Clinical dimensions obtained from SANS and SAPS. Item loadings after VARIMAX rotation. Bold type indicates the item loadings that contribute to each dimension (an item loading is counted into a specific dimension if its value >0.4).

		Dimension					
		1 Affective flattening and alogia	2 Auditory, somatic and visual hallucinations, and first rank and paranoid delusions	3 Formal thought disorders	4 Avolition and social isolation	5 Bizarre behaviour	6 Olfactory hallucinations and other delusions
Eigenvalue		12.5	7.3	3.7	2.04	1.9	1.5
% of variance		25.1	14.7	7.3	4.1	3.7	3
Items	Facial expression	**0.80**	0.05	0.003	0.37	0.03	0.004
	Spontaneous movements	**0.83**	0.01	–0.04	0.23	0.02	–0.09
	Expressive gestures	**0.84**	0.04	–0.05	0.34	0.01	0.01
	Eye contact	**0.79**	0.04	0.09	0.20	0.17	–0.10
	Affective non-responsiveness	**0.79**	0.04	0.001	0.34	0.09	0.005
	Vocal inflections	**0.84**	–0.009	0.02	0.27	0.07	0.11
	Poverty of speech	**0.74**	–0.09	0.13	0.19	0.35	–0.03
	Poverty of content	**0.64**	–0.10	0.31	0.27	0.24	0.10
	Blocking	**0.49**	0.09	0.33	–0.08	0.25	0.05
	Latency of response	**0.71**	–0.02	0.14	0.07	0.27	–0.13
	Grooming and hygiene	0.38	0.07	0.13	0.34	**0.42**	0.17
	Impersistence at work	0.23	–0.01	0.04	**0.60**	0.20	0.08
	Physical anergia	0.37	0.08	0.07	**0.64**	0.01	–0.04
	Recreational interests	0.36	0.16	0.07	**0.71**	0.073	–0.04
	Sexual interest	0.38	0.05	0.02	**0.66**	0.01	0.05
	Intimacy and closeness	0.38	0.12	0.11	**0.67**	0.15	0.07
	Relationship with friends	0.37	0.11	0.08	**0.72**	0.03	0.03
	Social inattentiveness	**0.49**	0.001	0.34	0.33	**0.42**	0.02
	Inattentiveness during testing	0.30	–0.05	0.26	0.29	**0.46**	0.03
	Auditory hallucinations	–0.13	**0.72**	0.06	0.24	0.16	0.07
	Voices commenting	–0.20	**0.75**	0.11	0.16	0.09	–0.03
	Voices conversing	–0.08	**0.75**	0.16	0.15	0.08	0.09
	Somatic hallucinations	0.03	**0.51**	0.12	0.18	0.13	0.41
	Olfactory hallucinations	0.01	0.29	0.05	0.26	0.06	**0.48**
	Visual hallucinations	–0.02	**0.50**	0.06	0.12	0.19	0.39
	Persecutory delusions	–0.07	**0.75**	0.04	0.16	0.17	0.12
	Delusions of jealousy	0.08	0.05	–0.03	–0.12	0.16	**0.55**
	Delusions of guilt	–0.12	0.08	0.02	0.0001	0.12	**0.46**
	Grandiose delusions	–0.05	0.08	0.06	0.03	0.05	**0.61**
	Religious delusions	0.002	0.19	0.04	–0.02	–0.09	**0.68**
	Somatic delusions	0.002	0.37	0.17	0.15	0.07	**0.52**
	Delusions of reference	–0.07	**0.71**	–0.04	0.09	0.17	0.11
	Delusions of being controlled	0.06	**0.77**	0.19	0.01	0.06	0.16
	Delusions of mind reading	0.14	**0.78**	0.06	–0.08	–0.004	0.12
	Thought broadcasting	0.14	**0.75**	0.07	–0.13	–0.01	0.07
	Thought insertion	0.13	**0.78**	0.04	–0.03	0.004	0.13
	Thought withdrawal	0.15	**0.77**	0.06	–0.14	–0.06	0.10
	Clothing and appearance	0.24	0.21	0.23	–0.12	**0.57**	0.07
	Social and sexual behaviour	0.22	0.25	0.17	0.08	**0.59**	0.15
	Aggressive behaviour	0.09	0.26	0.02	0.13	**0.49**	0.20
	Stereotyped behaviour	0.19	0.11	0.05	0.21	**0.54**	0.29
	Derailment	0.26	0.21	**0.74**	0.08	0.01	0.22
	Tangentiality	0.27	0.16	**0.66**	0.15	0.06	0.26
	Incoherence	0.27	0.04	**0.76**	–0.01	0.08	0.11
	Illogicality	0.25	0.14	**0.71**	0.02	–0.005	0.19
	Circumstantiality	–0.19	0.14	**0.68**	–0.04	0.05	–0.07
	Pressure of speech	–0.26	0.10	**0.60**	0.09	0.04	0.04
	Distractible speech	0.16	–0.04	**0.61**	0.16	0.33	–0.06
	Clanging	–0.06	0.04	**0.54**	0.07	0.26	–0.15
	Inappropriate affect	0.17	0.09	**0.48**	–0.05	0.51	–0.06

Table 4. QTDT analysis of clinical dimensions for 17 SNPs in the *NOS1AP* gene region

SNP	Number[a]	Inf. trios	Dim. 1	Dim. 2	Dim. 3	Dim. 4	Dim. 5	Dim. 6
rs12090585	1	47	*	*	*	*	*	*
rs1538018	4	40	*	*	*	*	*	*
rs945713	5	44	*	*	*	*	*	*
rs1415263	7	43	*	0.034 (C)	*	*	*	*
rs3924139	8	38	*	*	*	*	*	*
rs4592244	9	38	*	*	*	*	*	*
rs4145621	11	39	*	*	*	*	*	*
rs6680461	13	34	*	*	*	0.004 (G)	*	*
rs4657181	14	45	*	*	*	*	*	*
rs10800405	15	31	*	*	*	0.001 (G)	*	*
rs2661818	16	32	*	*	*	*	*	*
rs3751284	17	50	0.016 (C)	*	*	*	*	*
rs1504430	18	42	*	*	*	*	*	*
rs17468951	19	44	*	*	*	*	*	*
rs12122048	20	39	*	*	*	*	*	*
rs905720	21	43	*	*	*	*	*	0.048 (C)
rs11806859	24	31	*	*	*	*	*	*

Overall Bonferroni significance level: 0.10; overall empirical significance level: 0.08

[a] SNP number as in figure 1. Inf. trios., number of informative trios for the marker; Dim., dimension. For the explanation of the dimensions, see text and table 3. p values are shown for each marker/dimension combination; the allele that increases the trait (dimension) value is shown in parentheses. * p value >0.05.

Haplotype Association

Haplotype analyses were carried out based on the two LD regions shown in table 1 and figure 2a. The results of association tests for LD region 1 (markers rs945713 [#5] to rs2661818 [#16]) are shown in table 2. The omnibus haplotype test resulted in a p value of 0.35, indicating no significant overall association of this region with schizophrenia. In the haplotype-specific test, the p value for the most common haplotype was 0.04 with an odds ratio of 0.54, indicating a possible trend towards (negative) association between this particular haplotype and schizophrenia. For five of seven markers in LD region 1, the haplotype showing possible negative association consisted of alleles found to be undertransmitted in the single marker analyses (rs945713 [#5] – A; rs3924139 [#8] – C; rs4145621 [#11] – T; rs6680461 [#13] – T; and rs4657181 [#14] – T). No significant association was detected between LD region 2 (markers rs1504430 [#18] to rs348624 [#22]) and schizophrenia (data not shown).

QTDT Analysis on Clinical Dimensions

Principal component factor analysis identified six clinical dimensions accounting for 57.9% of the phenotypic variance: (1) affective flattening and alogia; (2) auditory, somatic and visual hallucinations, and first rank and paranoid delusions; (3) formal thought disorders; (4) avolition and social isolation; (5) bizarre behaviour, and (6) olfactory hallucinations and other delusions. Individual items and their factor loadings are listed in table 3.

The results of the QTDT analysis on these clinical dimensions are summarised in table 4. Since the QTDT program can only test markers for which 30 or more probands are informative, markers rs11579080 [#2], rs1572495 [#3], rs4657179 [#10], rs348624 [#22], and rs1123005 [#23] could not be included in the analysis. Nominally significant associations were obtained for the following marker-dimension combinations (the allele associated with increased trait values is given after the p value): dimension 1 (affective flattening and alogia) with marker rs3751284 [#17] (p = 0.016; C), dimension 2 (auditory, somatic and visual hallucinations, and first rank and paranoid delusions) with marker rs1415263 [#7] (p = 0.034; C), dimension 4 (avolition and social isolation) with markers rs6680461 [#13] (p = 0.004; G) and rs10800405 [#15] (p = 0.001; G), and dimension 6 (olfactory hallucinations and other delusions) with marker rs6680461 [#13] (p = 0.048; C). Three of the markers showing nominally significant p-values in the analysis of

clinical dimensions had also shown nominally significant p values in the categorical analyses (rs3751284 [#17], rs1415263 [#7], and rs6680461 [#13]). For markers rs3751284 [#17] and rs1415263 [#7], the alleles associated with increased trait values in the quantitative analysis are different from the ones shown to be overtransmitted to schizophrenic patients in the categorical analysis (tables 1 and 4), whereas for marker rs6680461 [#13], the allele shown to increase the trait value corresponds to the overtransmitted allele in the categorical analysis (allele G).

Discussion

NOS1AP is an interesting candidate gene for schizophrenia susceptibility because its product, the NOS1AP protein, seems to be involved in NMDA receptor mediated glutamatergic neurotransmission, which is thought to be implicated specifically in negative symptoms in schizophrenia [10, 42]. Intracellular NMDA receptor induced signal transmission relies on the interaction of the receptor molecule with the neuronal Nitric Oxide Synthase (nNOS) through a mediator protein, PSD95. NOS1AP competes with PSD95 for interaction with nNOS and is thought to be involved in the regulation of nNOS activity in the neuron [43]. Dysregulation of NOS1AP availability could therefore lead to a disruption of signalling processes following glutamatergic neurotransmission downstream of the NMDA receptor. In line with a possible role for abnormal NOS1AP expression in schizophrenia, Xu and colleagues have recently identified a short isoform of the NOS1AP protein and shown its increased expression in the schizophrenic brain in comparison to healthy subjects in a post-mortem brain study [44].

The observation of association of several SNPs in the *NOS1AP* gene to the schizophrenia phenotype and also to specific clinical dimensions of the disorder confirms and extends our previous results with microsatellite marker D1S1679 [16] and further strengthens the link between *NOS1AP* and schizophrenia.

Of the SNPs found to be associated with schizophrenia in our study, two were also found to be significant in the study by Brzustowicz and colleagues: rs1415263 [#7] and rs4145621 [#11] [11]. Moreover, the associated allele was the same in both studies: the T allele of rs1415263 [#7], and the C allele of rs4145621 [#11] (table 1). Interestingly, Xu and colleagues [44] found the expression levels of the short *NOS1AP* isoform overexpressed in the schizophrenic brain to be associated with the T allele of marker rs1415263 [#7].

The only statistically significant SNP outside LD region 1, rs3751284 [#17], is a synonymous change in exon 6 of the gene. The markers in LD region 1 showing association are located in intron 2 of *NOS1AP*, and it is possible that these markers are in LD with a further, unidentified susceptibility-conferring variant, such as a regulatory element upstream of the *NOS1AP* gene. This scenario could also explain the identification of a protective, rather than a risk-conferring, haplotype – the common protective haplotype 1 might be in LD with the protective allele at the susceptibility locus, whereas the risk allele at the same locus might be in LD with not one, but several of the remaining, rarer haplotypes, thereby diluting the susceptibility-conferring effect in the observed haplotypes. Another possible explanation for our findings is that intronic and synonymous exonic SNPs could affect posttranscriptional mRNA processes [45]. In both scenarios, genetic variation could lead to changes in the availability of functional NOS1AP in the neuron and thereby to alterations in glutamatergic neurotransmission.

The analysis of clinical dimensions showed an association between markers rs6680461 [#13] and rs10800405 [#15] and features of dimension 4 (avolition and social isolation), which captures negative symptoms of the disorder. For both markers, the allele associated with increased symptom severity is also overtransmitted to schizophrenic patients in the single marker categorical TDT analysis (although the results do not reach statistical significance for rs10800405 [#15]; see tables 1 and 3). These findings are consistent with a role of NOS1AP in the NMDA receptor pathway and the NMDA receptor hypofunction theory of the negative syndrome of schizophrenia [10, 42].

For dimension 1 (affective flattening and alogia), the C allele of marker rs3751284 [#17] was found to increase trait values. However, in the categorical single marker analysis, this allele was found to be undertransmitted to schizophrenic patients. In a similar manner, the C allele of marker rs1415263 [#7] is associated with increased values for dimension 2 (auditory, somatic and visual hallucinations, and first rank and paranoid delusions) but was found to be undertransmitted to schizophrenic patients. The p values from the quantitative analyses are only marginallyally significant, particularly in view of the many tests carried out in this analysis. For marker rs905720 [#21], the direction of association is the same in both categorical and quantitative analyses. However, the categorical results are not significant, and the uncorrected p value in the quantitative analysis is only marginally signifi-

cant. Further studies are needed to shed light on a possible role of *NOS1AP* in dimensions 1, 2 and 6.

While there is mounting evidence for a possible link between *NOS1AP* and schizophrenia, not all results are consistent. A large case-control study by Puri and colleagues failed to replicate the positive findings in a British sample [17]. Instead, these authors have found an association between schizophrenia and the *UHMK1* gene, also located on chromosome 1q23, in the British sample and they suggested that the original linkage signal, as well as the subsequent association results for *NOS1AP* by Brzustowicz and colleagues might be due to *UHMK1* [46]. However, the analysis of LD patterns in the European Hapmap population in the region encompassing *NOS1AP* and *UHMK1* revealed no significant LD between these two genes (data not shown). Antioquian nuclear DNA has a highly predominant European ancestry, and LD patterns in Europeans are proxy for LD patterns in Antioquia, as seen in figure 2. We therefore conclude that our results are not due to LD between *NOS1AP* and *UHMK1*.

In conclusion, we have found significant association of markers within the *NOS1AP* gene to schizophrenia and to a clinical dimension capturing negative symptoms of the disorder (avolition and social isolation). Our results are consistent with previous studies conducted in different populations and provide further evidence for the implication of the *NOS1AP* gene in the aetiology of schizophrenia. Future work should aim to investigate whether this association, particularly the association of *NOS1AP* with negative symptoms of the disease. To shed further light on a possible role of *NOS1AP* in the deficit syndrome, it would be of special interest to focus follow-up studies on deficit patients.

Acknowledgements

This work was partly funded by Colciencias grant 1115-04-11944 (Colombia), CODI-Universidad de Antioquia grants E-00810 and CIM-E01242 (Colombia) and grant R01 MH62440 from the National Institutes of Mental Health (USA). We would like to thank Mari-Wyn Burley for technical assistance and Gonçalo Abecasis for advice on the use of his QTDT programme. We are particularly grateful to all the patients and family members who participated in this study.

URLs

GENEPOP homepage: http://wbiomed.curtin.edu.au/genepop/

The HapMap project: http://www.hapmap.org/

QTDT homepage: http://www.sph.umich.edu/csg/abecasis/QTDT/

WHAP homepage: http://pngu.mgh.harvard.edu/~purcell/whap/index.shtml

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