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Family-based association study of common variants, rare mutation study and epistatic interaction detection in *HDAC* genes in schizophrenia

Oussama Kebir [a,b,c], Boris Chaumette [a,b,c], Mar Fatjó-Vilas [d,e], Amirthagowri Ambalavanan [f], Nicolas Ramoz [g], Lan Xiong [f], Fayçal Mouaffak [b,c,h], Bruno Millet [b,c,i], Nematollah Jaafari [c,j,k,l], Lynn E. DeLisi [m], Douglas Levinson [c,n], Ridha Joober [o], Lourdes Fañanás [d,e], Guy Rouleau [f,p], Caroline Dubertret [g,q], Marie-Odile Krebs [a,b,c,*]

[a] Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine Paris Descartes, Service Hospitalo-Universitaire, Centre Hospitalier Sainte-Anne, Paris, France
[b] INSERM, U894, Laboratory "Pathophysiology of Psychiatric Disorders", Centre of Psychiatry and Neurosciences, Paris, France
[c] Institut de Psychiatrie-GDR 3557 de Psychiatrie, France
[d] Departament de Biologia Animal, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain
[e] Institut de Biomedicina de la Universitat de Barcelona (IBUB), Centre for Biomedical Researh Network on Mental Health (CIBERSAM), Instituto de Salud Carlos III, Madrid, Spain
[f] Centre of Excellence in Neuromics of Université de Montréal, CHUM Research Center, Montreal, Quebec H2L 4M1, Canada
[g] INSERM, U894, Team "Vulnerability of Psychiatric and Addictive Disorders", Centre of Psychiatry and Neurosciences, Paris, France
[h] Service de Psychiatrie, Hôpital Bicêtre, Le Kremlin-Bicêtre, France
[i] Pôle Hospitalo-Universitaire de Psychiatrie Adulte, CH Guillaume Régnier, Rennes, France
[j] Unité de recherche clinique intersectorielle en psychiatrie du Centre Hospitalier Henri Laborit, Poitiers, France
[k] INSERM CIC-P 1402, CHU de Poitiers, France
[l] INSERM U 1084 Experimental and Clinical Neurosciences Laboratory, Université de Poitiers, France
[m] VA Boston Healthcare System, Harvard Medical School, Brockton, MA, USA
[n] Stanford University School of Medicine, Stanford, CA, USA
[o] Department of Psychiatry, McGill University, Montreal, Canada
[p] Montreal Neurological Institute and Hospital, Department of Neurology and Neurosurgery, McGill University, Montreal, Canada
[q] Assistance Publique-Hôpitaux de Paris, Hôpital Louis Mourier, Psychiatry Department, Colombes, France

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ABSTRACT

Background: Histone deacetylases (HDACs) are key enzymes of histone acetylation, and abnormalities in histone modifications and in the level of HDAC proteins have been reported in schizophrenia. The objective of the present study was to systematically test the *HDAC* genes for its association with schizophrenia.
Methods: A family-based genetic association study (951 Caucasian subjects in 313 nuclear families) using 601 tag-single nucleotide polymorphisms in *HDAC* genes was conducted followed by a replication study of top-ranked markers in a sample of 1427 Caucasian subjects from 241 multiplex families and 176 trios. Epistasis interaction was tested by using the pedigree-based generalized multifactor dimensionality reduction (GMDR). Furthermore, we analyzed exome sequencing data of 1134 subjects for detection of rare mutations in *HDAC* genomic regions.
Results: In the exploratory study, ten markers were in significant association with schizophrenia ($P < 0.01$). One maker rs14251 (*HDAC3*) was replicated ($P = 0.04$) and remained significant in the whole sample ($P = 0.004$). GMDR identified that a significant three-locus interaction model was detected involving rs17265596 (*HDAC9*), rs7290710 (*HDAC10*) and rs7634112 (*HDAC11*) with a good testing accuracy (0.58). No rare mutations were found associated with schizophrenia.
Conclusion: This first exploratory systematic study of the *HDAC* genes provides consistent support for the involvement of the *HDAC3* gene in the etiology of schizophrenia. A statistical epistatic interaction between *HDAC9*, *HDAC10*, and *HDAC11* was detected and seems biologically plausible.

1. Introduction

Schizophrenia is a complex neurodevelopmental disorder, influenced by both genetic and environmental factors. The characterization of these genetic predisposing factors has proven challenging (Gejman et al., 2010; Keshavan et al., 2008) partly because environmental factors modulate the penetrance of gene variants. Epidemiological, clinical, and

* Corresponding author at: INSERM, U894, Laboratory "Pathophysiology of Psychiatric Disorders", Centre of Psychiatry and Neurosciences, 2 ter rue d'Alésia, 75014 Paris, France. Tel.: +33 1 45658646; fax: +33 1 45658160.
E-mail address: marie-odile.krebs@inserm.fr (M.-O. Krebs).

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molecular features associated with psychosis support the hypothesis that epigenetic mechanisms are indeed implicated in the etiology of schizophrenia, including the incomplete concordance between monozygotic twins, fluctuating disease courses, sexual dimorphism, lifespan peaks of susceptibility and the episodic course of disease (Pidsley and Mill, 2011). Several molecular mechanisms can mediate epigenetic regulation, including DNA methylation, and post-translational modifications of histones (Berger, 2007): acetylation, methylation and phosphorylation. In general, increased histone acetylation is associated with increased transcriptional activity, whereas decreased acetylation is associated with decreased gene expression. Histone deacetylases (HDACs) play a role in the steady-state levels of acetylation of the core histones as opposed to histone acetyltransferases. In humans, the HDAC family can be divided into three distinct phylogenetic classes: class I (HDACs 1, 2, 3, and 8), class II (HDACs 4, 5, 6, 7, 9, and 10) and class III, which includes the sirtuin deacetylases that are structurally and functionally different from classical HDACs (Dokmanovic et al., 2007). Class II HDACs are sub-classified into class IIa (HDACs 4, 5, 7 and 9) and class IIb (HDACs 6 and 10) based on their primary structure and tissular distribution (Verdin et al., 2003). Finally, HDAC11, more recently described, has been proposed to represent class IV (Dokmanovic et al., 2007).

Several studies suggest the involvement of HDAC dysfunction in major psychotic disorders. First, mRNA expression levels of *GAD67* in the prefrontal cortex of patients with schizophrenia were found to be strongly and negatively correlated with mRNA expression levels of *HDACs 1*, *3*, and *4* (Sharma et al., 2008). Second, HDAC activity has been found to be enhanced in the prefrontal cortex of patients with schizophrenia (Akbarian et al., 2005). Third, the use of HDAC inhibitors such as valproate was found to be effective in inducing DNA demethylation and facilitating chromatin remodeling in a preclinical model of psychosis (Guidotti et al., 2011). Last, some molecular variability at the genes encoding for HDACs has been associated with psychosis and neurodevelopmental disorders. A case–control study of a Korean population reported that one SNP of *HDAC4* was associated with schizophrenia (Kim et al., 2010) and a deletion in *HDAC9* has also been reported in schizophrenia (Tam et al., 2010; Lang et al., 2012). In addition, *HDAC4* haploinsufficiency and mutations were identified in brachydactyly-mental retardation (BDMR) syndrome, which includes autistic symptoms (Williams et al., 2010).

We hypothesized that mutations in the exomic sequences of HDAC genes and/or common genetic variability in the *HDAC* genes could modify the activity of the protein, leading to suboptimal histone acetylation of candidate genes. Since the HDAC enzymes interact with other HDACs to form a functional complex, we further hypothesized that epistatic interactions between *HDAC* at-risk genetic polymorphisms could significantly increase the risk of psychosis. We have conducted a systematic family-based association study of the classical *HDAC* genes in schizophrenia, using tag-SNPs in a set of 325 trios followed by a replication study of the top-ranked makers in 241 multiplex families and 176 trios. Our findings were compared with the results of the mega-analysis of population-based association studies deposited in the Psychiatric Genomics Consortium (PGC) database. Also, we explored whether rare mutations in *HDAC* genes could play a role in psychosis risk increase by examining exome sequences of these regions in a sample of 108 patients, 142 related controls, and 884 unrelated controls. Finally, the top markers of the *HDAC* genes were subjected to an epistatic interaction study using generalized multifactor dimensionality reduction method.

2. Methods and materials

All participants gave their written informed consent. The study was performed in accordance with the guidelines of the institutions involved and was approved by the respective ethics committees of each participating center. All procedures were carried out according to the Declaration of Helsinki. Subjects were evaluated by a trained psychiatrist using a semi-structured interview that assesses the DSM-IV criteria for lifetime diagnosis and the diagnosis of schizophrenia or schizoaffective disorder was confirmed in the probands using DIGS 3.0 (Diagnostic Interview for Genetic Studies (Nurnberger et al., 1994)) or CASH (Comprehensive Assessment of Symptoms and History (Andreasen et al., 1992) for the "Barcelona" cohort). Exclusion criteria included: major medical illnesses that could affect brain function, substance-induced psychotic disorder, neurological conditions, history of head trauma with loss of consciousness, and moderate or severe mental retardation. Genomic DNA was extracted from peripheral blood cells or buccal mucosa (in some samples of Barcelona cohort) using standard methods.

2.1. Family-based exploratory association study

2.1.1. Subjects and DNA samples

Families were included in this study based on the presence of one proband diagnosed with schizophrenia or schizoaffective disorder. The global sample included 951 Caucasian subjects in 313 nuclear families (325 cases and their 626 parents forming 325 case–parent trios). It was composed of four samples: (a) *The REFAPSY cohort*: 274 subjects in 88 nuclear families (98 cases and 176 parents forming 98 case–parent trios) (multi-center enrollment, France), (b) *The "Colombes" cohort*: 182 subjects in 60 nuclear families (62 cases and 120 parents forming 62 case–parent trios) (Paris, France), (c) *The "Barcelona" cohort*: 411 subjects in 137 nuclear families (137 cases and 274 parents forming 137 case–parent trios) (multi-center enrollment, Spain), and (d) *The "Montreal" cohort*: 84 subjects in 28 nuclear families (28 cases and 56 founders forming 28 case–parent trios) (Montreal, Canada).

Demographic and clinical characteristics, as well as inclusion criteria and diagnostic screening methods used to exclude probands with other axis 1 disorders, have been previously reported for each cohort (Fathalli et al., 2008; Mouaffak et al., 2011; Fatjó-Vilas et al., 2011; Dubertret et al., 2010).

2.1.2. Selection and genotyping of SNPs

Ten classical *HDAC* genes were considered (*HDACs 1*, *2*, *3*, *4*, *5*, *6*, *8*, *9*, *10*, and *11*) (see genomic context in Supplemental information 1). SNPs were selected by using the tag-SNP method with $r^2 = 1$. Only SNPs with a minor allele frequency >5% according to HapMap Caucasian (CEU) data were considered. The covered regions included the 10 genes of interest as well as the 10 kb region at both 3′ and 5′ UTR of each gene. Selection was enriched by all known missense SNPs in these genes. A total of 642 SNPs were submitted to Illumina for iSelect custom BeadChips® genotyping. 601 SNPs were successfully genotyped (average SNP call rate > 92%) and were subjected to quality control analyses.

2.2. Family-based replication association study

2.2.1. Subjects and DNA samples

The replication study included 1427 subjects from two independent cohorts: (a) 976 subjects from 241 multiplex Caucasian families recruited in USA (DeLisi et al., 2002); (b) 451 individuals forming 176 trios of Caucasian origin recruited in Canada, Hungary and Tunisia (Fathalli et al., 2008).

2.2.2. Genotyping of SNPs

We used TaqMan genotyping assays (Applied Biosystems, Carlsbad, CA) to genotype the top SNPs associated in the exploratory study (only nine SNPs of ten have TaqMan probes at the moment of the replication study).

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2.3. PGC data

We extracted case–control association findings from the PGC (https://pgc.unc.edu/Sharing.php#SharingOpp) for the top *HDAC* SNPs associated with schizophrenia in our exploratory study using the tool Ricopili (http://www.broadinstitute.org/mpg/ricopili/).

2.4. Exome variations in HDAC family of genes

We considered the regions for all the *HDAC* genes in a total of 1134 exomes and the variant calling was performed using Genome Analysis Tool Kit (GATK) as part of the S2D project detailed elsewhere (Gauthier et al., 2010; Tarabeux et al., 2011). Out of these total numbers of exome, 884 exomes were unrelated controls from a cohort of non-psychiatric background, 108 exomes were subjects with schizophrenia, and 142 exomes for related controls (i.e. unaffected parents and siblings). Rare variant analysis of the pathogenicity was performed by using validated online software (Polyphen2.0, Exome Variant Server, SIFT).

2.5. Statistical analysis

2.5.1. Family-based association studies

Quality control of genotyping data was performed using PLINK v1.07 (Purcell et al., 2007). SNPs were used for analysis only if they met the following criteria: (a) greater than 90% of attempted genotypes were successful; (b) parental alleles were in Hardy–Weinberg equilibrium (0.001 threshold); (c) minor allele frequency was greater than 5%; and (d) there was no detected Mendelian inheritance error. Single-marker analyses were performed by applying the transmission/disequilibrium test (TDT) using PLINK. They were also performed using FBAT under both the additive and recessive models (Laird et al., 2000). Parent of origin tests were performed using PLINK. Haplotype blocks, defined by the criteria of Gabriel et al. (2002), were determined using HAPLOVIEW v4.2 (Barrett et al., 2005). Global (omnibus) haplotype tests of association were performed using UNPHASED v3.1.4 (Dudbridge, 2008). Only haplotypes with a frequency higher than 5% and an omnibus and haplotypic test *P*-value < 0.01 were considered.

2.5.2. Epistatic interaction

We merged the exploratory and the replication samples (n = 2378; 1048 cases; 730 families). Gene–gene interactions of *HDAC* genes were analyzed using a nonparametric and genetic model-free alternative to logistic regression based on data-mining approach: the generalized multifactor dimensionality reduction (GMDR) method (v1.0) (Lou et al., 2008) with the pedigree-based method II (5 cross validations and 10,000 permutations). Testing accuracy (TA), Z-score, and *P*-value were calculated for each model. We considered only interaction models with a TA superior to 0.54 and a significant Bonferroni-corrected *P*-value.

3. Results

3.1. Exploratory association study

3.1.1. Single marker analysis

In the exploratory study, a total of 551 SNPs remained after quality control and were included in the familial association analyses. An overview of their distribution in the selected genes is given in Supplemental information 2. Using the TDT, 59 autosomal SNPs had nominal *P*-values < 0.05. Among them, 10 SNPs showed significance levels at $P < 0.01$ (Table 1). The best association was found for rs7290710, located in *HDAC10* locus (OR: 1.46, CI 95%: 1.15–1.84, $P = 0.0015$). Three other SNPs in significant association (rs1076649, rs5771109 and rs742184) were also located in the *HDAC10* locus. Three SNPs (rs1726596, rs12531908 and rs7801662) encompass the *HDAC9* gene. One SNP is

Table 1
Association results of *HDAC* SNPs with schizophrenia in the exploratory study (n = 325 trios) and in the replication study (n = 417 families).

SNP	Gene	Alleles[a]	MAF		HWE *P*		TDT[b]				FBAT recessive model					
							Transmitted/untransmitted			Transmitted/untransmitted			Allele	N families	*P*	N families
			Exploratory	Replication	Exploratory	Replication	Exploratory	X^2	*P*	Replication	X^2	*P*		Exploratory		Replication
rs7290710	*HDAC10*	G/A	0.37	0.39	0.49	0.217	172/118	10.06	0.0015	142/129	0.99	0.32	G	183	0.0063	122
rs14251	*HDAC3*	C/A	0.42	0.43	0.51	0.124	115/168	9.92	0.0016	127/159	4.04	0.04	C	151	0.0036	120
rs7634112	*HDAC11*	C/A	0.17	0.16	0.04	0.011	118/75	9.58	0.0019	96/86	0.33	0.56	C	153	0.0085	121
rs1076649	*HDAC10*	G/A	0.37	0.35	0.09	0.24	118/169	9.06	0.0026	136/150	0.27	0.60	G	177	0.0169	76
rs1726596	*HDAC9*	G/A	0.09	0.12	0.08	0.636	62/33	8.85	0.0029	58/73	1.38	0.24	G	81	0.0009	77
rs12531908	*HDAC9*	A/G	0.29	0.28	0.49	1	151/105	8.26	0.0040	117/118	0.06	0.80	G	178	0.0004	49
rs5771109	*HDAC10*	A/G	0.38	0.40	0.79	0.349	173/124	8.08	0.0044	106/98	0.22	0.63	G	185	0.0177	65
rs7801662	*HDAC9*	C/A	0.39	0.38	0.09	0.622	190/139	7.90	0.0049	139/127	0.59	0.44	C	193	0.0265	118
rs17038236	*HDAC11*	G/A	0.10	0.10	0.82	0.613	42/71	7.44	0.0063	66/50	3.84	0.05	G	97	0.0017	76
rs742184	*HDAC10*	G/A	0.30	NA	0.85	NA	116/161	7.31	0.0068	NA	NA	NA	G	192	0.0180	NA

FBAT: family based association test; *HDAC*: histone deacetylase genes; HWE: Hardy–Weinberg equilibrium; MAF: minor allele frequency; SNP: single nucleotide polymorphism; TDT: transmission disequilibrium test.
[a] Major/minor alleles.
[b] For replication study: parental TDT.

located in HDAC3 (rs14251) and two in HDAC11 (rs7634112 and rs17038236). FBAT analyses confirm the association of these 10 markers with the disease. The TDT and FBAT recessive model data for the 59 SNPs is shown in Supplemental information 3.

3.1.2. Haplotype association analysis

Three small haplotypes (up to 2 kb) located in the *HDAC3*, *HDAC10* and *HDAC11* genes, showed significant association with schizophrenia (haplotype and omnibus tests $P < 0.01$). Haplotype blocks are shown in Supplemental information 4 and the most frequently associated haplotypes are shown in Supplemental information 5.

3.2. Replication study

Only one marker from the top-ranked SNPs of the exploratory study was replicated: rs14251 (*HDAC3*) (parental TDT: $P = 0.044$; FBAT recessive model: $P = 0.017$) (Table 1). When the two samples are merged, rs14251 was significantly associated with schizophrenia (n = 2378; 1048 cases; 730 families; $P = 0.0038$; 326 transmissions/242 non transmissions; OR = 1.35, CI 95%: [1.13–1.59]) (Table 2).

3.3. Parent of origin effect

In order to test for a preferential transmission of parental alleles, we used the explicit 'Parent of origin' test. None of the 10 markers in significant association with schizophrenia showed a significant difference in transmission from the maternal and the paternal lineage in the whole sample.

3.4. PGC data

We examined if the top-ranked SNPs from the exploratory study were in association with schizophrenia in the PGC database. The data were available for all SNPs but one (rs7290710) and, the findings were not significant (Supplementary information 6). Also, we screened PGC eQTL data in the brain and the blood (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014): rs14251 and rs11742646 were not associated with eQTL.

3.5. Exome sequencing analysis of HDAC regions

The analysis of variants showed that there were 5 non-synonymous variants, causing changes in the protein (Table 3). One rare variant was found exclusively in patients, but SIFT and Polyphen analyses predicted that it would not be damaging.

Table 2
Replication association results of top *HDAC* SNPs in the whole sample (n = 2378; 1048 cases; 730 families).

SNP	Gene	Alleles[a]	*P* (parental TDT)	
			Unadjusted	Bonferroni correction
rs7290710	*HDAC10*	G/A	0.0058	0.0529
rs14251	*HDAC3*	C/A	0.0004	0.0038
rs7634112	*HDAC11*	C/A	0.0071	0.0645
rs1076649	*HDAC10*	G/A	0.0065	0.0587
rs1726596	*HDAC9*	G/A	0.3861	1
rs12531908	*HDAC9*	A/G	0.0373	0.3357
rs5771109	*HDAC10*	A/G	0.0136	0.1225
rs7801662	*HDAC9*	C/A	0.0136	0.1229
rs17038236	*HDAC11*	G/A	0.3903	1

HDAC: histone deacetylase genes; SNP: single nucleotide polymorphism; TDT: transmission disequilibrium test.
[a] Major/minor alleles.

3.6. Epistatic interaction detection

Using the GMDR method, we examined the gene–gene interaction for all two-, three-, and four-locus models between the most significant SNPs for each *HDAC* highlighted by the exploratory study: rs7290710 (*HDAC10*), rs14251 (*HDAC3*), rs7634112 (*HDAC11*) and rs1726596 (*HDAC9*). Table 4 shows the different interactive models. The three-locus interaction model was the most significant model at the experiment-wide level and involved SNPs in the *HDAC9, HDAC10* and *HDAC11* genes ($P = 0.004$). This result remained significant after Bonferroni correction ($P = 0.044$). Testing accuracy value was high (0.58), which indicates that this model under the selected high-risk/low-risk parameterization performs well in correctly classifying individuals.

4. Discussion

The present study investigated the association between classical *HDAC* genes and schizophrenia with a comprehensive approach: an exploratory family-based association study using tag-SNPs, a replication family-based study of the obtained top SNPs, an extraction of mega-analysis data of the PGC regarding these markers, an analysis of an exome sequencing database regarding regions of *HDAC* genes, and finally an epistatic interaction detection study of the most significant *HDAC* genes in the total sample. Rare mutations were found in patients and controls; all of them were qualified as non-damaging. This negative result does not provide support for role HDAC mutations in schizophrenia, although larger samples could be needed to identify rare variants. By contrast, a common variant in *HDAC3* region and epistatic interactions between *HDAC9, HDAC10* and *HDAC11* genes were found associated with schizophrenia. Analyses conducted in the exploratory sample (n = 951, 325 families) followed by the replication study (n = 1427, 417 families) identified a signal in the 5′ UTR region of *HDAC3* gene. One marker, rs14251, initially detected ($P = 0.0017$) was replicated ($P = 0.044$) and it is significantly associated with schizophrenia in the whole sample.

HDAC3 is a small gene (about 16 kb) very closely neighbored by adjacent genes. The marker rs14251 is precisely located in *RELL2*, coding for the RELT-like protein 2 specifically expressed in human immune-related tissues, and also located in *FCHSD1*, coding for the FCH and double SH3 domains protein 1. For these two partially overlapping 5′ adjacent genes to *HDAC3*, rs14251 is reported as a missense variant and is predicted as benign by Polyphen. Both encoded proteins have no precise function and their role in behavioral disorders is unknown. More probably, the functional consequence of rs14251 polymorphism could result or be in linkage disequilibrium with a polymorphism that results in a change in 5′ UTR sequence of *HDAC3* gene, a domain that is classically admitted as critical for translation regulation. One possibility is that this genetic polymorphism might alter the binding of transcription factors to DNA or the spatial conformation of DNA at this locus leading to a suboptimal HDAC enzymatic activity. HaploReg resource (Ward and Kellis, 2012) indicates that rs14251 changes the interaction with *INSM1* (insulinoma-associated protein 1) which contains five zinc-finger motifs and was described as a transcription factor playing a role in early embryonic neurogenesis (Lan and Breslin, 2009). SCANdatabase (Gamazon et al., 2010) was used to predict trans-regulatory effects of rs14251. Interestingly, it could alter *SHANK2* (SH3 and multiple ankyrin repeat domains 2) expression through affecting its antisense RNA regulation. *SHANK2* is one of the major genes implicated in autism and schizophrenia (Guilmatre et al., 2014).

HDAC3 enzyme is expressed in the brain, in particular in the nucleus and the cytoplasm of monoaminergic and neuropeptidergic neurons (Takase et al., 2013). Previously, the *HDAC3* gene has been found to correlate negatively with GAD67 level, a critical enzyme for GABA synthesis in the prefrontal cortex of subjects with schizophrenia (Sharma et al., 2008). While HDAC3 seems to be present at the crossroad of many

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Table 3
List of *HDAC* rare variants in exome sequencing.

Chr	Position	Ref allele	Mut allele	Variant class	HDAC	Variant function type	Exonic/coding variant type	Mutation	Total affected with variant	Total controls with variant	Exome variant server	SIFT score	Polyphen score
12	48179178	C	T	SNP	7	Exonic	nonsynonymous_SNV	p.R891Q	2	1	0.000077	0.1	0.002
12	48189753	G	A	SNP	7	Exonic	nonsynonymous_SNV	p.P281L	5	0	na	0.12	0.073
12	48196036	C	G	SNP	7	Exonic	nonsynonymous_SNV	p.G14A	1	1	na	0.16	0.423
22	50686900	C	T	SNP	10	Exonic splicing	nonsynonymous_SNV	p.G303D	1	1	na	na	0.999
7	18674271	C	G	SNP	9	Exonic	nonsynonymous_SNV	p.S273C	1	1	na	0.02	na

Chr: chromosome; ref allele: allele of reference; mut allele: mutant allele; HDAC: name of the HDAC gene; na: not applicable; SNV: single nucleotide variant.

neurobiological processes, beside its role in regulating the acetylation levels of the core histones, its precise function is still incompletely known. However, recent finding reports have implicated it in long-term memory formation (McQuown et al., 2011). Focal deletion or inhibition of *HDAC3* gene in CA1 area of the dorsal hippocampus in mice resulted in enhancement of long-term memory for object location. Moreover, forced expression of HDAC3 protein in mice leads to neuronal death (Bardai and D'Mello, 2011) but has no effect in other cell lines. Thus, the HDAC3 protein seems to play a neurotoxic role and has also been shown to promote neurodegeneration. HDAC3 operates in interaction with HDAC1 and a truncated form of HDAC9 inhibits HDAC1–HDAC3 interaction to prevent this neurotoxic effect (Bardai et al., 2012). Genetic polymorphism in any of these enzymes might impair HDAC complex formation and/or prevent optimal interactions that lead to negative functional consequences. In mice, subchronic administration of antidepressants, mood-stabilizers and antipsychotics induced a significant increase of HDAC3 protein expression in the striatum, nucleus accumbens, hippocampus, cingulate cortex, and amygdala (Ookubo et al., 2013). Moreover, HDAC3 expression was reported to increase after delta9-THC administration in a dose-dependent manner (Khare et al., 2006). Finally, HDAC3 is downregulated in the environmental chronic mild stress model in mice (Tordera et al., 2011).

Very few studies have exhaustively studied genetic polymorphisms of the *HDAC* genes for association with psychiatric disorders. An unpublished study by Wedenoja (2010) reported that rs11742646 located in the 5′ region of *HDAC3* was significantly associated with Stroop performance in schizophrenia in a large set of Finnish families ($P = 0.0004$). Interestingly, this marker is in strong LD ($r^2 = 0.97$) with the replicated marker rs14251 in our study and has a *P*-value of 0.026. The haplotype formed by rs14251–rs11742646 was also significant (haplotypic test: $P = 0.016$, omnibus test: $P = 0.0099$). Another intronic SNP of *HDAC3* (rs2735188) was reported to be in suggestive association with autism in a genome-wide scan including 1558 families ($P = 0.00002$) (Anney et al., 2010). Interestingly, this marker has a significance of 0.0202 in our sample. Altogether, these results suggest that common variants of *HDAC3* can play a role in vulnerability schizophrenia and autism, probably by means of affecting cognitive processes. We are aware of only one published study that investigated common genetic variants in *HDAC* genes for association with schizophrenia. Kim et al. (2010) reported that rs1063639 in *HDAC4* was associated with schizophrenia ($P = 0.016$) in a case–control study in a Korean population (278 patients vs. 234 controls). In our exploratory study, this marker was not significant. In PGC mega-analyses, none of the top SNPs was significant. This lack of replication could be explained by the genetic heterogeneity of samples composing PGC database, which could lead to a loss of signal, as well as ethnical heterogeneity and methodological differences regarding statistical association. Given that HDAC proteins interact and form multiprotein complexes (Joshi et al., 2013), and that a HDAC protein seldom operates alone, we tested the hypothesis that epistatic interaction between polymorphisms of *HDAC* genes may confer an increased risk of schizophrenia. In human pathology, some epistatic interactions between *HDAC* genes coding were reported: *HDAC1* and *HDAC3* polymorphisms interact and represent a prognostic indicator in patients with hepatocellular carcinoma (Yang et al., 2010). A three-order epistatic interaction between *HDAC9*, *HDAC10* and *HDAC11* genes was found. This finding obtained in a large family sample is robust and survives after stringent Bonferroni correction. It is biologically plausible since specific interaction between HDAC9 and HDAC10 enzymes was reported (Kotian et al., 2011). Furthermore, all of HDAC9, HDAC10, and HDAC11 proteins are specifically co-expressed in the dopaminergic and noradrenergic neurons (Takase et al., 2013) but not in the neuropeptidergic or serotoninergic neurons. HDAC9 seems to be expressed in mature neurons (Lang et al., 2012) and could regulate dendritic growth of cortical neurons (Sugo et al., 2010). Finally, HDAC11 is also expressed in oligodendrocytes and might play a role during central nervous system development (Liu et al., 2008).

5. Conclusion

In summary, we report a significant and replicated association of a *HDAC3* 5′ UTR marker (rs14251) with schizophrenia using a family-based design. We also report a statistical epistatic interaction between

Table 4
Best interactive model for 4 *HDAC* genes associated with schizophrenia on the basis of GMDR in the whole sample.

No. of loci	SNPs	Effective sample	Training accuracy	Testing accuracy	Standard-error of testing accuracy	*P* (Z-score)	*P* (Z-score) after Bonferroni correction
2	rs7290710–rs14251	1109	0.554	0.492	0.025	0.785	1
	rs7290710–rs7634112	1107	0.549	0.480	0.027	0.764	1
	rs7290710–rs1726596	1083	0.553	0.524	0.027	0.191	1
	rs14251–rs7634112	1132	0.558	0.540	0.025	0.103	1
	rs14251–rs1726596	1098	0.550	0.468	0.026	0.942	1
	rs7634112–rs1726596	1102	0.563	0.519	0.025	0.446	1
3	rs7290710–rs14251–rs7634112	1097	0.581	0.480	0.027	0.873	1
	rs7290710–rs14251–rs1726596	1074	0.577	0.510	0.028	0.370	1
	rs7290710–rs7634112–rs1726596	1073	0.602	0.580	0.026	0.004	0.048
	rs14251–rs7634112–rs1726596	1088	0.595	0.557	0.028	0.085	0.936
4	rs7290710–rs14251–rs7634112–rs1726596	1064	0.633	0.553	0.027	0.056	0.618

HDAC: histone deacetylase genes; SNP: single nucleotide polymorphism; GMDR: generalized multifactor dimensionality reduction; no. of loci: number of loci interaction.

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HDAC9, *HDAC10* and *HDAC11* genes as a genetic risk factor for schizophrenia. By contrast, our results do not provide support for a role of *HDAC* mutations in schizophrenia. The present study is the first comprehensive exploration of the role of the *HDAC* genes in schizophrenia and further studies are needed to investigate the functional correlates of the detected associations with regard to the epigenetic mechanisms involved in psychosis pathophysiology.

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The sponsors had no role in the design and conduct of the study; collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Contributors

OK, BC, GR and MOK designed the study. LF, GR and MOK obtained funding and supervised the study. FM, BM, NJ, LED, RJ, LF and CD collected the data. OK, BC, AA, NR and LX analyzed the data. OK, BC, MFV, NR, LX, DL and RJ interpreted the data. OK, BC, MFV, NR, CD and MOK drafted the report. All authors contributed to and have approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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