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SHORT COMMUNICATION

Analysis of complete mitochondrial genomes of patients with schizophrenia and bipolar disorder

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The present study aims at investigating the association between common and rare variants of mitochondrial DNA (mtDNA), and increased risk of schizophrenia (SZ) and bipolar disorder (BPD) in a cohort of patients originating from the same Italian population. The distribution of the major European mtDNA haplogroups was determined in 89 patients and their frequencies did not significantly differ from those observed in the Italian population. Moreover, 27 patients with high probability of having inherited the disease from the maternal side were selected for whole mitochondrial genome sequencing to investigate the possible presence of causative point mutations. Overall, 213 known variants and 2 novel changes were identified, but none of them was predicted to have functional effects. Hence, none of the sequence changes we found in our sample could explain the maternal component of SZ and BPD predisposition.
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INTRODUCTION

Schizophrenia (SZ) and bipolar disorder (BPD) are among the top ten causes of disability worldwide.[1] Despite extensive genetic and pharmacological studies, the etiology and pathophysiology of these mental disorders are still unknown, and the nuclear susceptibility loci identified so far can explain only a small fraction of the genetic component of these diseases.[2]

The growing body of observations published in the last decade points to the involvement of mitochondria in the pathophysiology of psychiatric disorders, including SZ and BPD.[3] Several genetic studies reported association between these diseases and common mitochondrial DNA (mtDNA) polymorphisms defining ethnic-specific mitochondrial haplogroups.[4–5] Other analyses found new rare variants with a putative functional effect[6] and a global excess of synonymous substitutions in the dorsolateral prefrontal cortex of SZ patients.[7] Despite the great interest of these findings, the role of mtDNA in the pathogenesis of SZ/BDP remains unclear.

The present study aims at investigating the association between common and rare variants of mtDNA and the increased risk of SZ/BPD in a sample of patients originating from the same Italian population.

MATERIALS AND METHODS

Samples

All analyzed subjects originated from the population of Chioggia, a North-East Italian town. All patients were diagnosed according to DSM-IV criteria as described previously.[8] Most patients belong to complex pedigrees in which the segregation of SZ or BPD, as well as the co-segregation of both phenotypes, have been observed. This is in agreement with the hypothesis of a genetic overlap between SZ and BPD; in this study, we hence considered both SZ and BPD cases. Overall, the sample included a total of 89 patients belonging to different maternal lineages; among them, 35 were sporadic and the other 54 were extracted from 41 complex pedigrees (for further details see Supplementary Materials and Methods).

Mt-Haplogroup analysis and mtDNA genome resequencing

We investigated the distribution of mtDNA haplogroups (mt-hgs) in the 89 SZ and BPD patients by combining restriction fragment length polymorphism analysis, with sequencing of hypervariable sequence I (HVS-I) of the mitochondrial displacement-loop. The most common European mt-hgs were classified using single-nucleotide polymorphisms previously reported.[9] In addition, a 466-bp fragment encompassing the HVS-I was sequenced in all samples. Sequence variants were determined between mtDNA nucleotides 16001 and 16400 by comparison with the revised Cambridge Reference Sequence (rCRS; GenBank accession no. NC_012920.1).

The complete mt-genome was sequenced using the GeneChip Mitochondrial Resequencing Array 2.0 (MitoChip v.2.0, Affymetrix, Santa Clara, CA, USA) following the manufacturer's protocol. Sequencing process, data acquisition and statistical analyses were conducted as described in the Supplementary Materials and methods. The haplogroup classification was based on the phylogeny proposed by van Oven and Kayser,[10] and reported at http://www.phylotree.org/ (mtDNA tree Build 11).

RESULTS AND DISCUSSION

We used our data to reconstruct the phylogenetic relationships among patients' mtDNAs. The variations found in the HVS-1 region of the

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89 patients are reported in Supplementary Table 1. The most frequent European haplogroups, that is, H, V, K, U, J, T, I, W and X, accounted for 90% of the genetic variability observed in our sample (Table 1). Haplogroup distribution analysis did not reveal a disease susceptibility to mt-hgs; all major European mt-hgs were represented in the sample and no significant differences in their overall distribution were observed when compared with those reported for three Italian control samples (Table 1).[11–13] The frequencies of each mt-hg in patients and controls were then analyzed by applying the Bonferroni's adjustment for multiple comparisons and no statistically significant differences were observed.

These findings confirmed a previous study that had failed to identify SZ susceptibility mt-hgs on Italian patients.[11] However, these negative results could be due to the small size of our sample, which would allow to detect only large changes in haplogroup frequency distribution.

The lack of power for association with a mtDNA haplogroup, however, is not necessarily incompatible with the identification of mtDNA variations, as many mitochondrial diseases are due to recurrent mutations.[14] We therefore sequenced the complete mtDNA of 27 patients, searching for susceptibility mtDNA mutations. Among this group, 22 individuals were familiar cases belonging to pedigrees with either mother-to-child transmission in more than one generation or with all-affected siblings in the sibship (Supplementary Figure 1). The remaining five were sporadic cases, who shared the same mt-hg and HVS-1 sequence with one of the other selected cases (for details see Supplementary Table 1).

All the mtDNA variations detected in the 27 patients are shown in the phylogenetic tree in Figure 1. The mtDNA sequencing of the sporadic cases revealed that only the two X2b samples (39.1, 52.1) share the same mtDNA sequence. As hg X2b is characterized by an infrequent occurrence in the Italian population (2.9%),[15] our data suggest that the two subjects were related in the recent past, and a deeper investigation is required to ascertain their possible cryptic relatedness. In addition to the two X2b subjects, only three other samples (21.2, 22.1 and 16.1), belonging to mt-hg H1b, shared exactly the same mtDNA sequence. Interestingly, these samples also shared variant m.8348A>G with another sample of our collection (4.5) and with the Italian H1b sample reported by Achilli *et al.*[16] This result suggests that variant 8348 is diagnostic for a sub-clade of the H1b typical of the Italian population, as it had not been described until now in other samples. Among our cohort, we sequenced two patients (one familiar and one sporadic) belonging to mt-hg N1a. Even though they differ from each other for two mutations in the coding regions, they both possess the 16147G allele, which defines the African/South Asian branch of the N1a haplogroup,[17] and have lost the canonical N1a polymorphism at position 16248. Such findings make the sequences peculiar within the N1a lineage. Moreover, these two samples shared the mutations at position 151 and 2758 with a sample of African origins published by Gonder *et al.*,[18] thereby suggesting that 151 and 2758 are good candidates to be markers of a new sub-clade inside the N1a haplogroup.

Considering the 27 sequenced mt-genomes, a total of 215 substitution events, including 148 in the coding region (positions 577–16023), were observed. Most of these variants have been already described in the literature and only two of them (Table 2) are not reported in any public database. These latter are two synonymous changes identified at position 7666 and 8590 in the *ATPase6* and *COII* genes, respectively. The new mutations were observed in single patients: no recurrent susceptibility mtDNA mutations occurring multiple times in different mt-hgs and peculiar to our sample were identified. Altogether, these

Table 1 mtDNA haplogroup distribution in SZ/BPD patients and in three independent samples of the Italian population

	Patients			*Italian CT1*[a]				*Fisher's exact test*	*Italian CT2*[b]			*Fisher's exact test*	*Italian CT3*[c]			*Fisher's exact test*
Hg	N=*89*	*(%)*	*CI (95%)*	N=*190*	*(%)*	*CI (95%)*	P-*values*	N=*1486*	*(%)*	*CI (95%)*	P-*values*	N=*775*	*(%)*	*CI (95%)*	P-*values*
H	35	39.33	29.1–50.2	77	40.53	33.5–47.9	0.9	646	43.47	40.9–46.0	0.51	311	40.13	36.7–43.7	0.91
V	5	5.62	1.8–12.6	—	—	—	—	—	—	—	—	29	3.74	2.5–5.3	0.38
K	7	7.87	3.2–15.4	13	6.84	3.7–11.4	0.80	114	7.67	6.4–9.1	0.68	68	8.77	6.9–11	1
U	8	8.99	4.0–16.9	18	9.47	5.7–14.6	1.00	186	12.52	10.9–14.3	0.41	109	14.06	11.7–16.7	0.25
J	6	6.74	2.5–14.1	19	10.00	6.1–15.2	0.50	126	8.48	7.1–10.0	0.69	62	8.00	6.2–10.1	0.84
T	8	8.99	4.0–16.9	26	13.68	9.1–19.4	0.33	143	9.62	8.2–11.2	1.00	85	10.97	8.9–13.4	0.72
I	5	5.62	1.8–12.6	4	2.11	0.6–5.3	0.15	36	2.42	1.7–3.3	0.08	14	1.81	1–3	0.04*
W	1	1.12	0.03–6.1	7	3.68	1.5–7.4	0.29	—	—	—	—	16	2.06	1.2–3.3	1
X	5	5.62	1.8–12.6	—	—	—	—	—	—	—	—	15	1.94	1.1–3.2	0.05
N	3	3.37	0.7–9.5	—	—	—	—	—	—	—	—	9	1.16	0.5–2.2	0.11
Other	6	6.74	2.5–14.1	26[d]	13.68	9.1–19.4	0.12	235[e]	15.81	14.0–17.8	0.01	57[f]	7.35	5.6–9.4	1

Abbreviations: BPD, bipolar disorder; CI, confidence interval; SZ, schizophrenia.
Overall mt-hg distributions comparison: our patients versus Italian CT1 control sample: $P=0.52$; our patients versus Italian CT2 control sample: $P=0.29$; our patients versus Italian CT3 control sample: $P=0.08$.
*This difference in the frequency of hgI did not remain significant after correction for multiple comparisons (*P*-value cut-off=0.004).
[a]This sample is the control group reported by Magri *et al.*[11]; it consists of 190 subjects living in northern Italy.
[b]This sample is the CT1 group reported by Ghezzi *et al.*[12]; it consists of 1486 subjects of general Italian ancestry.
[c]This sample is the control group reported by Santoro *et al.*[13]; it consists of 775 controls mainly collected in the north of Italy.
[d]This class of CT1 also includes hgX, hgV and hgN.
[e]This class includes the "Other" and "L-M" classes of Ghezzi *et al.*[12]
[f]This class includes the "HV", "L", "M", "ROa", "R1" and "Other" classes of Santoro *et al.*[13]

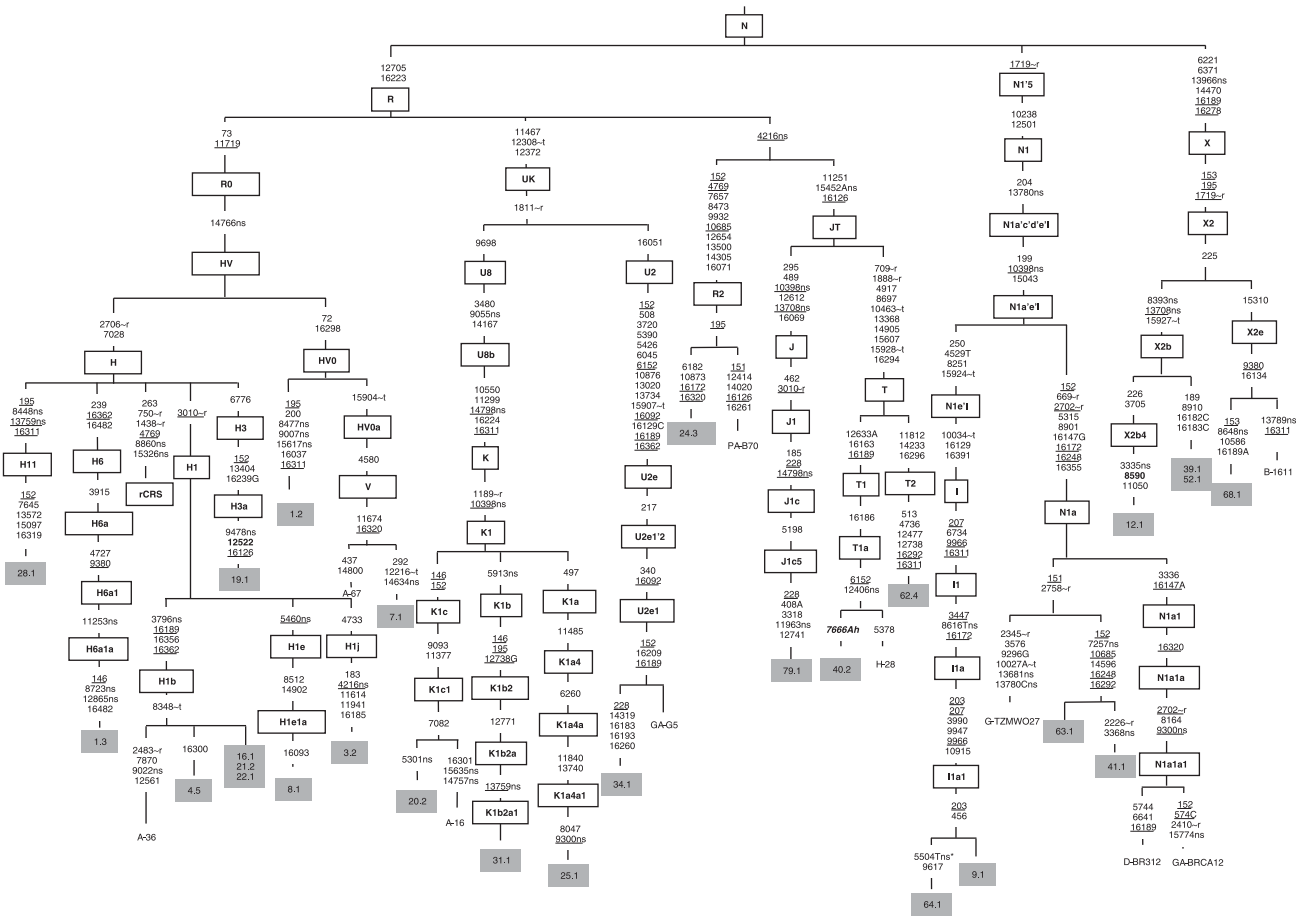

Figure 1 Phylogenetic tree of 38 complete mtDNA sequences. Eleven complete sequences were taken from the literature and we referred to these particular samples as A,[16,24] B,[25] D,[26] GA,[27] G,[18] H[28] or PA,[29] followed by '−' and the original sample code (for further details see Supplementary Materials and methods). Mutations are shown on the branches; they are transitions, unless the base change is explicitly indicated. 'h' following the nucleotide position indicates heteroplasmy; ns denotes non-synonymous mutations; ∼r and ∼t indicate mutations in the rRNA and tRNA genes, respectively; recurrent mutations are underlined; boldface mutations are those reported here for the first time; grey boxes contain patients identification number. Insertions and deletions are not reported, as well as variants at position 16519, as it is a known mutation hotspot.

Table 2 New sequence variants[a] identified in the present study

Position	*Base change*	*Locus*	*Amino-acid change*
7666	C>A	*COII*	Synonymous
8590	C>T	*ATPase6*	Synonymous

Each mutation was identified in one patient.
[a]The novelty of each sequence variant was determined by using the on-line Mitomap database (http://www.mitomap.org/MITOMAP), the mtDNA database (http://www.ianlogan.co.uk/mtDNA.htm), the Human Mitochondrial Genome Database (http://www.mtdb.igp.uu.se/), the database of 5140 human mitochondrial genomes reported in the work of Pereira *et al.*,[30] and by using the web-based search approach described by Bandelt *et al.*[31]

data suggest that our new mutations are unlikely to contribute to the susceptibility to SZ in our sample, even though their functional effect cannot be definitely ruled out.

Lastly, we hypothesized that mild deleterious mutations in mtDNA could contribute to susceptibility to SZ and BPD, potentially uncoupling the OXPHOS activity. For this reason, we compared the ratio of nonsynonymous/synonymous substitutions in the mt-genes calculated in our sample to that reported in the general population.[19] No statistically significant differences were observed, considering either the entire coding region or each mt-gene separately (Supplementary Table 2).

Most of the mitochondrial disease-related mutations have been detected in the tRNA genes and they mainly affect the secondary structure of the molecule.[20] We have identified 10 variants in tRNA genes (Supplementary Table 3), but none of them is likely pathogenic, as they involve nucleotide positions that are <90% conserved in mammalian species.[21] The only exception is the 12308 transition, which falls in a tRNA-conserved region; however, it is not considered pathogenic, as it is diagnostic of haplogroups U and K, and an increased frequency among SZ patients of these two haplogroups have never been reported.

In our cohort, only one variant, the synonymous transversion at 7666 in the *COII* gene, was identified in a heteroplasmic state. However, only substantial heteroplasmy can be detected by the MitoChip v.2.0, and therefore, we might have missed other heteroplasmic mutations.[22] In this study, we did not attempt to address this issue, as somatic mtDNA variants acquired in the tissue or organs involved in the disease are usually not detected in the blood samples, which typically exhibit much less heteroplasmy than non-dividing tissues.[23]

In conclusion, our results indicate that the pattern of maternal inheritance observed in some families of our sample cannot be explained by point variations in the mtDNA sequence.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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