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Mitochondrial DNA HV lineage increases the susceptibility to schizophrenia among Israeli Arabs

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Abstract

Haplotypes and haplogroups are linked sets of common DNA variants, acting as susceptibility or protective factors to complex disorders. Growing evidence suggests that dysfunction of mitochondrial bioenergetics contributes to the schizophrenia phenotype. We studied mitochondrial DNA haplogroups in schizophrenia patients. Since mitochondria are inherited from the mothers, we used healthy fathers as an ideal case-control group.

Analysis of the distribution of mitochondrial haplogroups in schizophrenia patients compared to their healthy fathers (202 pairs) resulted in an over-representation of the mtDNA lineage cluster, HV, in the patients ($p=0.01$), with increased relative risk (odds ratio) of 1.8. Since mitochondrial DNA is small relative to nuclear DNA, a total mitochondrial genome analysis was possible in a hypothesis-free manner. However, mitochondrial DNA haplogroups are highly variable in human population and it will be necessary to replicate our results in other human ethnic groups.

Keywords: Schizophrenia; Mitochondria; mtDNA; Haplogroup; Association

1. Introduction

Due to intense ATP-consuming processes in the brain, a high level of brain energy supply is required, gained by aerobic glucose oxidation into CO_2 and water. Mitochondria are the providers of cellular energy, and more than 90% of the oxygen consumed by the brain is used by the mitochondria to generate ATP through oxidative phosphorylation (Sciamanna and Lee, 1993). Abnormal cellular energy metabolism due to mitochondrial dysfunction may lead to alterations in neuronal

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function, plasticity and brain circuitry. Such alterations may contribute to the cognitive and behavioral abnormalities of schizophrenia.

An emerging body of data suggests that impaired energy metabolism due to mitochondrial dysfunction plays a role in the pathophysiology of schizophrenia (Ben-Shachar, 2002). Patients with mitochondrial dysfunction showed a higher incidence of psychiatric presentations preceding the diagnosis of mitochondrial disease (Fattal et al., 2006). Deformation and reduction in mitochondrial number was found in the prefrontal, anterior limbic cortex and caudate nucleus of treated as well as medication free schizophrenia patients compared with healthy controls (Kung and Roberts, 1999; Uranova and Aganova, 1989). Postmortem brain samples from schizophrenia patients exhibited reduced OXPHOS complex activity in the frontal and temporal cortices and in the basal ganglia (Karry et al., 2004). Microarray and proteomics studies in schizophrenia patients revealed patterns of altered mitochondria-related gene expression (Glatt et al., 2005; Iwamoto et al., 2005; Middleton et al., 2002). Antioxidants, which are scavengers of mitochondria-produced reactive oxygen species (ROS), are reduced in first episode schizophrenia patients (Reddy et al., 2003) and their administration improved the patients' condition (Arvindakshan et al., 2003). Moreover, dysfunction in DISC (disrupted in schizophrenia)1, a nuclear gene localized to the mitochondria which carries schizophrenia-associated SNPs, alters mitochondrial structure (Callicott et al., 2005; James et al., 2004; Millar et al., 2005). Thus, mitochondrial dysfunction might be involved in the etiology of schizophrenia.

It has been shown that linked sets of mitochondrial DNA (mtDNA) genetic variants (haplogroups) alter human tendency to develop neuropsychiatric disorders (such as Parkinson's and Alzheimer's diseases) (Wallace, 2005). Several attempts have been made to assess association of certain mtDNA genetic variants with schizophrenia (Bandelt et al., 2005; Marchbanks et al., 2003). However, those attempts focused on specific variants rather than on a hypothesis-free approach.

Here we show evidence for association of mutations in the mitochondrial genome defining the HV lineage cluster with the propensity to develop schizophrenia in an Israeli-Arab population.

2. Materials and methods

2.1. Patient cohort

A total of 202 DNA samples from unrelated schizophrenia patients in addition to their parents, all of Israeli-Arab origin, were collected in two independent locations (Beer-Sheva Mental Health Center, Beer-Sheva and Emek Medical Center, Afula) after informed consent. The original protocol was approved by the Helsinki committee (Beer-Sheva & Emek). Since mtDNA is maternally transmitted, samples of the patients' psychiatrically healthy fathers were used as normal controls.

DNA was extracted from peripheral lymphocytes using standard techniques (Phenol-chloroform).

2.2. mtDNA haplogroups classification

Genotyping was conducted using PCR amplification and restriction enzyme analysis (restriction fragment length polymorphisms, RFLPs) for the relevant polymorphic sites, in a hierarchical approach, starting from the most prevalent lineages in the Israeli-Arab population, the HV lineage, followed by haplogroups H, L, U, K, J, T, N1, W, X, I, R, N non-R. The rest of the less prevalent haplogroups in this population were aggregated as "others" (Fig. 1) (Owen et al., 2004).

The list of single nucleotide polymorphisms (SNPs), primers, restriction enzymes, and PCR conditions are presented in Supplementary Tables 2 and 3.

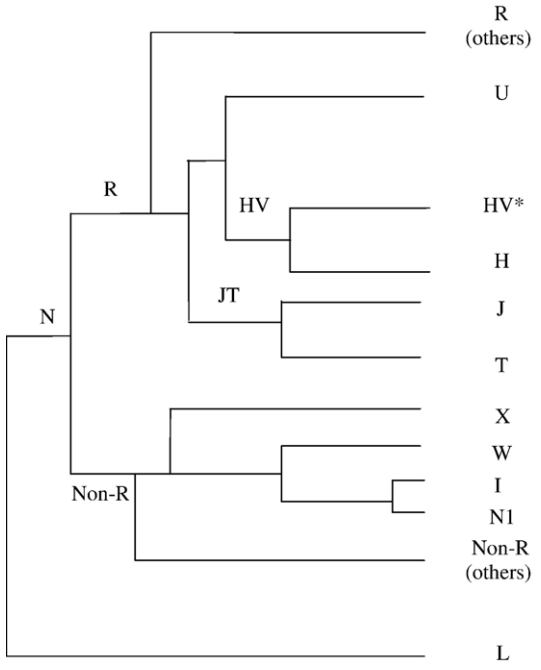

Fig. 1. A schematic representation of the phylogenetic topology of mtDNA lineages in the Israeli-Arab population.

2.3. HVR1 amplification and sequencing

In addition to the PCR RFLP analysis to assign the samples into haplogroups we also sequenced the hypervariable region 1 (HVR1) of the mtDNA control region in all the samples as previously described (Macaulay et al., 1999; Mishmar et al., 2003; Ruiz-Pesini et al., 2004). The HVR1 sequence is highly polymorphic and harbors multiple haplogroup-defining mutations, hence it assists in assigning samples to certain mtDNA haplogroups.

A 518-bp fragment encompassing the HVR1 region, located between mtDNA positions 15883 and 16401 was amplified with primers designed using the Oligos software (Molecular Biology Insight, Cascade, CO) and the PCR conditions described above. PCR products were purified with the ExoSAP-IT PCR clean-up kit (GE Healthcare Bio-Sciences Corp) and were sequenced by Danyel Biotech (Rehovot).

2.4. Data analysis

The sequences were aligned and manually checked using the Sequencher software (Gene Codes, Ann Arbor, MI) (Supplementary Table 4), and all polymorphic positions were confirmed by chromatograms.

2.5. Statistical analysis

To avoid small sample sizes, some of the haplogroups were grouped according to phylogenetic considerations: W and X (WX), I and N1 (N1). Samples that were not assigned to the more prevalent Caucasian haplogroups were assigned to the N macro-lineage and were divided into R and non R using the relevant SNPs (Supplementary Table 2). Samples that were not assigned to either L or any of the haplogroups comprising the N macro-lineage were aggregated as "others". Furthermore, to avoid small sample size the JT* haplogroup was also considered as "others" for the sake of the statistical analysis.

The statistical analyses were performed using Systat 9.0 (Systat Software, Inc., CA, USA). Results were regarded as statistically significant only if $\alpha < 0.05$. In order to test whether the susceptibility to schizophrenia (represented by a binary indicator variable taking on values 0 and 1) differs among haplogroups, logistic regressions were performed. Haplogroup is a categorical variable composed of 11 classes. Including this variable in a logistic regression model requires the employment of 10 indicator variables, *i.e.*, converting it into a dummy variable. The coefficients of these 10 indicator variables indicate whether the propensity to develop schizophrenia in each of the respective 10 haplogroups differs from that of the haplogroup that does not have an indicator variable (zero scoring in all 10 indicator variables). For simplicity, we present in the text analyses in which we treated the 10 haplogroups excluding the HV lineage (comprised of both the H and HV*) in the aggregate. To get an estimate for the relative risk of carriers of a particular haplogroup developing the disease, odds ratios (OR) were calculated.

3. Results and discussion

An analysis of 404 DNA samples of schizophrenia patients and their healthy fathers (202 pairs), all of Israeli-Arab origin, allowed the assignment of more than 90% of the patients and the healthy controls to certain mtDNA lineages. The population studied in the present work was originally collected as triads of probands and their parents and the cohort was used in several linkage and association studies of the nuclear genome (Dobrusin et al., 2001; Korostishevsky et al., 2006; Kremer et al., 2000). Since mitochondria are inherited from the mothers, we used healthy fathers as an ideal case-control group. We analyzed the mtDNA lineage distribution in patients and fathers and found that the HV lineage is 1.6 fold over-represented in the patients' group (Table 1). The HV lineage which is the dominant lineage in the Israeli-Arab population (Table 1) (Richards et al., 2003), is comprised of the H haplogroup and the lineage cluster HV*. Since both

Table 1
mtDNA haplogroup distribution in schizophrenia patients compared with healthy controls in Israeli Arabs

	Patients *N* (%)	Controls *N* (%)
H } HV lineage	41 (20.3)	27 (13.3)
HV* } HV lineage	19 (9.4)	10 (4.9)
L	12 (5.9)	12 (5.9)
U	22 (10.9)	26 (12.9)
K	11 (5.4)	14 (6.9)
J	13 (6.4)	19 (9.4)
T	12 (5.9)	10 (4.9)
JT**	1 (0.5)	4 (1.9)
N1	4 (1.9)	10 (4.9)
W	21 (10.4)	18 (8.9)
X	1 (0.5)	2 (0.9)
I	1 (0.5)	2 (0.9)
N non R	6 (2.9)	7 (3.4)
R	22 (10.9)	18 (8.9)
Others	16 (7.9)	23 (11.4)

*Samples of the HV lineage excluding haplogroup H.
**Samples of the JT lineage excluding haplogroups J and T.

H and HV* exhibited the same tendency of almost two-fold over-representation in the patients compared with the controls, we hypothesize that the HV lineage is responsible for these differences and the statistical analysis thus relates to the HV lineage. Logistic regression analysis using the entire HV lineage aggregate as a dummy variable, revealed a significant difference ($p=0.01$, Supplementary Table 2). This placed the linked set of mutations defining the HV lineage as a candidate risk factor to schizophrenia in Israeli Arabs, with an estimated odds ratio (OR) of 1.8 (1.14<95% CLI>2.90). Power analysis shows that a sample size of 222 subjects in each group would be required to replicate these results with a power of 80% and an alpha of 0.05 (two-tailed). Alternatively, since the replication would be based on an a-priori hypothesis, a sample size of 270 subjects in each group would be required for a power of 80% and an alpha of 0.01 (one-tailed).

The effect size of the difference in mtDNA haplogroup frequency found in the present study is comparable to other association studies of the mitochondrial genome. In Parkinson's disease it has been reported that the frequencies of haplogroup J (7.1% of the patients *vs.* 11.2% of the controls, OR 0.55, $p=0.02$) and K (5.6% of the patients *vs.* 9.4% of the controls, OR 0.52, $p=0.02$) are significantly lower in the patients compared with healthy controls (van der Walt et al., 2003), and Niemi et al. found significantly lower frequency of the HV cluster among the Finnish centenarian population compared with healthy controls (39.1% of the centenarians population *vs.* 55.0% of the controls, $p=0.001$) (Niemi et al., 2003). Thus, our findings imply that mitochondrial common variants alter the propensity to schizophrenia in a degree of significance comparable with other mitochondrial genome association reports. It may not be ruled out that in other ethnic populations different kinds of alterations in mtDNA haplogroup distribution are associated with schizophrenia.

The control group in the present study was comprised of the patients' psychiatrically healthy fathers, thus, it is unlikely that population stratification plays a confounding factor in this study. In addition, since all sampled fathers were older than the age of onset of schizophrenia, the chance of misdiagnosing the controls as healthy is significantly minimized. Yet, due to the obvious skew in gender distribution between patients and control samples we tested for possible heterogeneity in mtDNA lineage distribution between males and females in the patient group. As the distribution was not different between sexes ($G=3.28$, $df=5$, $p=0.65$) we pursued the patients *vs.* control comparisons without any correction for gender. It should be pointed out that since the controls are the fathers of the patients there was obviously no age matching and the possibility of an age effect on a subject's haplogroup may not be ruled out.

Previous reports suggested rare pathological mutations in the mitochondrial genome as possible causes of schizophrenia (Martorell et al., 2006), yet, to our knowledge, our study is the first whole mitochondrial genome association study using common mtDNA genetic variants as candidate susceptibility factors for schizophrenia. The present results are provisional and needed to be replicated in a larger sample.

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Funding for this study was provided by a Stanley Research Center grant to RHB. The Stanley foundation had no further role in study design, in the collection, analysis and interpretation of data, in the writing of the report, and in the decision to submit the paper for publication.

Contributors

Shirly Amar: designed the methodology to measure the different haplogroups and carried out the experiments and took an active and central part in the interpretation of the results and preparation of the manuscript. Alon Shamir: was pivotal in all details concerning the design of the methodology. Ofer Ovadia: was pivotal in the statistical analysis of the data. Monica Blanaru, Alon Reshef, Ilana Kremer, Marcella Rietschel, Thomas G. Schulze, Wolfgang Maier: played a pivotal role in patient diagnosis and blood collection. RH Belmaker and Richard Ebstein: contributed their expertise in psychiatric genetics. Galila Agam: instructed A. Shamir and S Amar in carrying out the lab work and contributed towards preparation of the manuscript. Dan Mishmar: is an expert in the mitochondrial genome and population genetics. He was pivotal in designing the methodology and the preparation of the manuscript.

Conflict of Interest

The authors state that they have no conflict of interest, financial or otherwise, with the publication of this manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.schres.2007.04.020.

References

Arvindakshan, M., Ghate, M., Ranjekar, P.K., Evans, D.R., Mahadik, S.P., 2003. Supplementation with a combination of omega-3 fatty acids and antioxidants (vitamins E and C) improves the outcome of schizophrenia. Schizophr. Res. 62 (3), 195–204.

Bandelt, H.J., Yao, Y.G., Kivisild, T., 2005. Mitochondrial genes and schizophrenia. Schizophr. Res. 72 (2-3), 267–269.

Ben-Shachar, D., 2002. Mitochondrial dysfunction in schizophrenia: a possible linkage to dopamine. J. Neurochem. 83 (6), 1241–1251.

Callicott, J.H., Straub, R.E., Pezawas, L., Egan, M.F., Mattay, V.S., Hariri, A.R., Verchinski, B.A., Meyer-Lindenberg, A., Balkissoon, R., Kolachana, B., et al., 2005. Variation in DISC1 affects hippocampal structure and function and increases risk for schizophrenia. Proc. Natl. Acad. Sci. U. S. A. 102 (24), 8627–8632.

Dobrusin, M., Corbex, M., Kremer, I., Murad, I., Muhaheed, M., Bannoura, I., Muller, D.J., Schulze, T.G., Reshef, A., Blanaru, M., et al., 2001. No evidence for linkage by transmission disequilibrium test analysis of microsatellite marker D22S278 and schizophrenia in a Palestinian Arab and in a German population. Am. J. Med. Genet. 105 (4), 328–331.

Fattal, O., Budur, K., Vaughan, A.J., Franco, K., 2006. Review of the literature on major mental disorders in adult patients with mitochondrial diseases. Psychosomatics 47 (1), 1–7.

Glatt, S.J., Everall, I.P., Kremen, W.S., Corbeil, J., Sasik, R., Khanlou, N., Han, M., Liew, C.C., Tsuang, M.T., 2005. Comparative gene expression analysis of blood and brain provides concurrent validation of SELENBP1 up-regulation in schizophrenia. Proc. Natl. Acad. Sci. U. S. A. 102 (43), 15533–15538.

Iwamoto, K., Bundo, M., Kato, T., 2005. Altered expression of mitochondria-related genes in postmortem brains of patients with bipolar disorder or schizophrenia, as revealed by large-scale DNA microarray analysis. Hum. Mol. Genet. 14 (2), 241–253.

James, R., Adams, R.R., Christie, S., Buchanan, S.R., Porteous, D.J., Millar, J.K., 2004. Disrupted in Schizophrenia 1 (DISC1) is a multicompartmentalized protein that predominantly localizes to mitochondria. Mol. Cell. Neurosci. 26 (1), 112–122.

Karry, R., Klein, E., Ben Shachar, D., 2004. Mitochondrial complex I subunits expression is altered in schizophrenia: a postmortem study. Biol. Psychiatry 55 (7), 676–684.

Korostishevsky, M., Kremer, I., Kaganovich, M., Cholostoy, A., Murad, I., Muhaheed, M., Bannoura, I., Rietschel, M., Dobrusin, M., Bening-Abu-Shach, U., et al., 2006. Transmission disequilibrium and haplotype analyses of the G72/G30 locus: suggestive linkage to schizophrenia in Palestinian Arabs living in the North of Israel. Am. J. Med. Genet. B Neuropsychiatr. Genet. 141 (1), 91–95.

Kremer, I., Rietschel, M., Dobrusin, M., Mujaheed, M., Murad, I., Blanaru, M., Bannoura, I., Muller, D.J., Schulze, T.G., Reshef, A., et al., 2000. No association between the dopamine D3 receptor Bal I polymorphism and schizophrenia in a family-based study of a Palestinian Arab population. Am. J. Med. Genet. 96 (6), 778–780.

Kung, L., Roberts, R.C., 1999. Mitochondrial pathology in human schizophrenic striatum: a postmortem ultrastructural study. Synapse 31 (1), 67–75.

Macaulay, V., Richards, M., Hickey, E., Vega, E., Cruciani, F., Guida, V., Scozzari, R., Bonne-Tamir, B., Sykes, B., Torroni, A., 1999. The emerging tree of West Eurasian mtDNAs: a synthesis of control-region sequences and RFLPs. Am. J. Hum. Genet. 64 (1), 232–249.

Marchbanks, R.M., Ryan, M., Day, I.N., Owen, M., McGuffin, P., Whatley, S.A., 2003. A mitochondrial DNA sequence variant associated with schizophrenia and oxidative stress. Schizophr. Res. 65 (1), 33–38.

Martorell, L., Segues, T., Folch, G., Valero, J., Joven, J., Labad, A., Vilella, E., 2006. New variants in the mitochondrial genomes of schizophrenic patients. Eur. J. Hum. Genet. 14 (5), 520–528.

Middleton, F.A., Mirnics, K., Pierri, J.N., Lewis, D.A., Levitt, P., 2002. Gene expression profiling reveals alterations of specific metabolic pathways in schizophrenia. J. Neurosci. 22 (7), 2718–2729.

Millar, J.K., James, R., Christie, S., Porteous, D.J., 2005. Disrupted in Schizophrenia 1 (DISC1): subcellular targeting and induction of ring mitochondria. Mol. Cell. Neurosci. 30 (4), 477–484.

Mishmar, D., Ruiz-Pesini, E., Golik, P., Macaulay, V., Clark, A.G., Hosseini, S., Brandon, M., Easley, K., Chen, E., Brown, M.D., et al., 2003. Natural selection shaped regional mtDNA variation in humans. Proc. Natl. Acad. Sci. U. S. A. 100 (1), 171–176.

Niemi, A.K., Hervonen, A., Hurme, M., Karhunen, P.J., Jylha, M., Majamaa, K., 2003. Mitochondrial DNA polymorphisms associated with longevity in a Finnish population. Hum. Genet. 112 (1), 29–33.

Owen, M.J., Williams, N.M., O'Donovan, M.C., 2004. The molecular genetics of schizophrenia: new findings promise new insights. Mol. Psychiatry 9 (1), 14–27.

Reddy, R., Keshavan, M., Yao, J.K., 2003. Reduced plasma antioxidants in first-episode patients with schizophrenia. Schizophr. Res. 62 (3), 205–212.

Richards, M., Rengo, C., Cruciani, F., Gratrix, F., Wilson, J.F., Scozzari, R., Macaulay, V., Torroni, A., 2003. Extensive female-mediated gene flow from sub-Saharan Africa into near eastern Arab populations. Am. J. Hum. Genet. 72 (4), 1058–1064.

Ruiz-Pesini, E., Mishmar, D., Brandon, M., Procaccio, V., Wallace, D.C., 2004. Effects of purifying and adaptive selection on regional variation in human mtDNA. Science 303 (5655), 223–226.

Sciamanna, M.A., Lee, C.P., 1993. Ischemia/reperfusion-induced injury of forebrain mitochondria and protection by ascorbate. Arch. Biochem. Biophys. 305 (2), 215–224.

Uranova, N.A., Aganova, E.A., 1989. Ultrastructure of the synapses of the anterior limbic cortex in schizophrenia. Zh Nevropatol Psikhiatr Im S S Korsakova 89 (7), 56–59.

van der Walt, J.M., Nicodemus, K.K., Martin, E.R., Scott, W.K., Nance, M.A., Watts, R.L., Hubble, J.P., Haines, J.L., Koller, W.C., Lyons, K., et al., 2003. Mitochondrial polymorphisms significantly reduce the risk of Parkinson disease. Am. J. Hum. Genet. 72 (4), 804–811.

Wallace, D.C., 2005. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu. Rev. Genet. 39, 359–407.