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Letter to the Editors

Mitochondrial genes and schizophrenia

Dear Editors,

Proving that a mitochondrial DNA (mtDNA) mutation is pathogenic is not a trivial task because mtDNA is highly polymorphic and some seeming polymorphism may be introduced through the sequencing process or incomplete digestion with restriction enzymes as an artifact. Several criteria assist in deciding whether a novel base change is pathogenic (Chinnery and Schon, 2003; DiMauro and Schon, 2001). Among these criteria, mutation in heteroplasmic state could easily be misused. The recent claim that a mitochondrial mutation associated with schizophrenia (Marchbanks et al., 2003) constituted such a case and should be received with some scepticism, especially in view of a number of published pitfalls where polymorphisms were obviously mistaken as pathogenic.

Marchbanks et al. (2003) designed a *Mse*I RFLP assay for a high throughput screening of the T12027C mutation in the ND4 gene they had discovered in heteroplasmic form in a postmortem brain of a patient with schizophrenia. They reported a frequent occurrence (81/181) of heteroplasmy at this position in different patients but also documented a considerable frequency of the "variant-rich fraction" in the control group (43/184). The latter finding, however, is significantly at variance with a large database of mtND4 sequences (Finnilä et al., 2001; Herrnstadt et al., 2002; Kong et al., 2003), where position 12027 is completely invariable and unambiguous (0/800; $p<0.001$). In their claim that T12027C played a significant role in schizophrenia, Marchbanks et al. (2003) evidently overlooked that there is a high risk of incomplete digestion by the restriction enzyme even with a 100% homoplasmic sample. In fact, there is a similar case on this point in the published resource, in which incomplete digestion by restriction enzyme took the camouflage role. Bidooki et al. (1997) firstly described heteroplasmic T10010C plus heteroplasmic A5656G in a patient with encephalomyopathy. Heteroplasmy of A5656G, however, can be an artifact of RFLP analysis (Finnilä et al., 1999). This mutation, previously observed by Thomas et al. (1996) was found to be in connection with A12308G (a marker for mtDNA haplogroup U), T3197C (a marker for haplogroup U5), and T5814C (which was associated with mitochondrial encephalopathy; Manfredi et al., 1996) in a patient with limb myopathy (Sternberg et al., 1998). Indeed, it is now well known that a transition at position 5656 (which is located as a non-coding spacer between the $tRNA^{Ala}$ and $tRNA^{Asn}$ genes) is characteristic of a sub-haplogroup (now referred to as U5b1; Tambets et al., 2004) of the European haplogroup U5b (Finnilä et al., 1999, 2001; Herrnstadt et al., 2002). Phylogenetic information is thus a useful guide to the evaluation of pathogeneity of a particular mutation, especially since the database of complete mtDNA sequences is rapidly growing; e.g. consult Mitomap (www.mitomap.org).

The preceding two cases demonstrate the importance of considering the mtDNA database in its entirety to evaluate the molecular basis of a mitochondrial disease. This is particularly important when a novel mutation is claimed to have been found. There are further instances that crop up in recent clinical studies. For example, Tzen et al. (2003) have identified a double mutation (a heteroplasmic A3243G and a homoplasmic A14693G) in a patient with MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes). Whereas the former mutation is well known to be associated with either

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MELAS or a wide array of disease phenotypes (ranging from diabetes and deafness to a mixture of chronic progressive external ophthalmoplegic symptoms and strokelike episodes), the transition A14693G has not been reported in clinical studies so far according to Mitomap - except for a (homoplasmic) occurrence in a patient with oculomotor myopathy (Sternberg et al., 2001). In this case, it was observed on a haplogroup H background along with the novel potentially pathogenic mutation G5698A (in heteroplasmic state). A14693G was also observed as a rare polymorphism in the Native American haplogroup D1 (Herrnstadt et al., 2002). In the recent version of the east Asian mtDNA tree, A14693G (together with three other coding region mutations, G8392A, A10398G, and T14178C) defines a whole branch, viz. haplogroup Y (Kong et al., 2003). This haplogroup is generally rather infrequent but has been spotted, e.g. in mainland China (Yao et al., 2002a) and also among the Han Chinese in Taiwan (Horai et al., 1996; Tsai et al., 2001). Incidentally, the data of Tzen et al. (2001) testify to a particular haplogroup Y lineage (as recognizable by G8392A) in Taiwan. We therefore affirm that A14693G should be regarded as a normal polymorphism rather than a deleterious mutation, as classified on the Mitomap website.

In short, to infer whatever association of a mutation with a certain disorder, but without functional assay such as cybrid analysis for confirmation, extensive sequencing of total mtDNA (rather than a quick RFLP analysis) in patients as well as controls would be needed. In any case, the phylogenetic position of the affected mtDNAs, given the current worldwide database of complete coding-region sequences, has to be taken into full consideration (cf. Rocha et al., 1999; Montiel-Sosa et al., 2002; Yao et al., 2002b; Kong et al., in press).

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