Letters to the editor

No association of ALDH2 genotype in MELAS

Dear Sir

Mitochondrial DNA encodes only for a limited number of mitochondrial proteins whereas the majority of the proteins which constitute the mitochondrion are of nuclear origin. Therefore, alterations in both mitochondrial and nuclear DNA have the potential to affect mitochondrial function [1].

Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) and mitochondrial diabetes mellitus associated with 3243 tRNA^{Leu(UUR)} mutation (MDM-3243) are both related to the same 3243 mutation [2]. The mechanism for the different association of the same mutation with the two diseases has not been clarified.

We previously reported the importance of inactive aldehyde dehydrogenase 2 (ALDH2) genotype, a nuclear DNA mutation, for inheritance of non-insulin-dependent diabetes mellitus (NIDDM) and its high prevalence in patients with MDM-3243 [3, 4]. This time, we investigated the genotype further in MELAS associated with the 3243 tRNA^{Leu(UUR)} mutation (MELAS-3243).

We recruited 34 MELAS subjects from four different institutes (National Institute of Neuroscience, Tohoku University, Saiseikai Central Hospital, and Showa University [16 male, 18 female, age from 4 to 50 years, mean $\pm$ SD: 23.4 ± 13.4 years, onset of MELAS from 3 to 44 years, 18.2 ± 12.2 years]). They were unrelated. They had hallmarks of MELAS, such as stroke, epilepsy, headache or convulsion. The 3243 mtDNA mutations and ALDH2 genotype were determined in peripheral leukocytes and/or in muscle. The details for the mitochondrial DNA and ALDH2 genotype examination have been described elsewhere [4–6]. The non-diabetic control group for ALDH2 genotype consisted of 461 Japanese who were the same as we previously reported elsewhere.

We found 11 (33%) patients with MELAS-3243 had the inactive ALDH2 genotype (Table 1). The frequency of the ALDH2 genotype in MELAS-3243 and normal control subjects were not different. This suggests that the inactive ALDH2 genotype is not associated with MELAS-3243 in contrast to the association of ALDH2 with MDM-3243 as we previously reported [4].

Corresponding author: Y. Suzuki, M. D., Saiseikai Central Hospital, 1-4-17, Mita, Minato-ku, Tokyo 108, Japan

Table 1. Genotype frequencies of ALDH2 in MELAS patients and normal control subjects

	Genotype frequency		
	ALDH2 ¹ / ALDH2 ¹	ALDH2 ¹ / ALDH2 ²	ALDH2 ² / ALDH2 ²
MELAS patients (<i>n</i> = 34)	0.68	0.33	0.00 ^a
Control subjects (<i>n</i> = 461)	0.58	0.35	0.07

Active ALDH2 (ALDH2¹/ALDH2¹) vs inactive ALDH2 (ALDH2¹/ALDH2² and ALDH2²/ALDH2²)

^a between MELAS and normal subjects; $\chi^2 = 1.2$

Odawara et al. [7] postulated that, because many MELAS families with 3243 mutation have diabetes and families with diabetes rarely complicate MELAS, factors other than the 3243 mutation are required to account for the phenotypes of MELAS. However, in general, MELAS-3243 patients have a high amount of 3243 mutation in tissues, while MDM-3243 patients show a very low percentage of the mutation even in tissues such as muscles [6]. Since the genetic contribution to pathogenesis in mitochondrial disease is supposed to be greater in those carrying large amounts of deleterious mutation MELAS-3243 may have a greater genetic contribution of 3243 mutation to the initiation of the disease than MDM-3243. In contrast, in MDM-3243, other factors like easy acetaldehyde accumulation due to inactive ALDH2 genotype or other unknown environmental factors may make a contribution in altering mitochondrial function [3, 4].

Thus, our results suggest the different association of ALDH2 genotype with MELAS-3243 and MDM-3243. This difference may give an hint as to the different aetiologies between the two diseases.

Yours sincerely,

Y. Suzuki, T. Muramatsu, M. Taniyama, Y.-i. Goto, Y. Oka, S. Suzuki, K. Tsukuda, Y. Atsumi, I. Nonaka, K. Hosokawa, A. Shimada, T. Asahina, K. Matsuoka

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Response from the author

Dear Sir,

The pathogenic mutation in mitochondrial DNA at position 3243 exhibits enigmatic properties when its phenotypic expression is considered. Many carriers of this mutation develop mitochondrial diabetes and deafness (MIDD). In contrast, other carriers of the 3243 mutation develop the severe neurological syndrome of MELAS [1, 2]. The data presented by Suzuki and collaborators in their letter and in their previous publication [3] indicate the association of inactive ALDH2 with the 3243 mutation in patients with mitochondrial diabetes. Remarkably, this association is absent in patients with MELAS. The authors suggest that the expected accumulation of acetaldehyde in carriers of inactive ALDH2 may be a factor in developing a diabetic phenotype. In addition, the authors propose that high heteroplasmy levels of the 3243 mutation may favour the development of MELAS. Though the data are interesting a note of caution is warranted with respect to genetic association studies. They are vulnerable to misinterpretation when the study groups are not fully matched for genetic background. The presented data are based on allele frequencies of mutant ALDH2 in a group of MELAS patients and a group of diabetic patients with the 3243 mutation. For comparison, the authors have included a large control group of non-3243 carriers. Because of the intrinsic situation of relatively small numbers of 3243 carriers in each group, which are obtained through clinics, it is difficult to find groups of 3243 carriers fully matched to the control group. Besides, spurious relationships may be present in selected patients from a specific geographical region. Despite this word of caution, the results by Suzuki and collaborators are certainly of interest and will trigger studies in other populations.

An aspect on which different points of view exist is whether variations in heteroplasmy levels alone are responsible for the development of the MELAS phenotype. Studies have shown clustering of MELAS in only some pedigrees with the 3243 mutation. Other studies found no clear relation between the phenotype and heteroplasmy levels and this has led to the hypothesis proposed by Odawara and Yamashita [4] that

additional factors are required for the development of MELAS. Heteroplasmy levels are generally determined on a population of cells in a tissue sample from patients. Studies by Matthews et al. [5] have provided evidence that there is variation of heteroplasmy between individual cells which is not adequately reflected by the mean heteroplasmy value for a particular tissue. These authors suggested that the distribution of the mutation over individual cells rather than the overall level of heteroplasmy may contribute to the development of the clinical phenotype. The existence of large differences in heteroplasmy levels between individual cells has been corroborated [6] by direct visualization of mutant mtDNA in individual cells by fluorescence in situ hybridization. It is my feeling that heterogeneity in heteroplasmy of the 3243 mutation at the level of individual cells has to be taken into consideration for the evaluation of phenotype-genotype relations.

Yours sincerely,

J. A. Maassen

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Corresponding author: Dr. J. A. Maassen, Department of Medical Biochemistry, Sylvius Laboratories, Wassenaarseweg 72, NL-2330 RA Leiden, The Netherlands