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CORRESPONDENCE

A 3-bp deletion of mitochondrial DNA tRNA^{Lys} observed in lymphoblastoid cells

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During the course of our studies to sequence mitochondrial DNA (mtDNA) in patients with bipolar disorder and in healthy control subjects,[1,2] we found a 3-bp deletion, m.8348–8350, in a lymphoblastoid cell line (LCL) of a healthy Japanese man (49-years old). This deletion occurred in the region of mitochondrial tRNA^{Lys} (Figure 1a). The deletion was not, however, detected in the lymphocyte DNA obtained from this subject (Figure 1a). Allele-specific polymerase chain reaction, which can detect a 0.1% mutation of total mtDNA, did not reveal the 3-bp deletion in the lymphocytes (Figure 1b). This finding suggests that the acquisition of this deletion occurred during formation of the LCL, although the possibility of a bottleneck effect cannot be ruled out.

Genetic instability of LCLs has been reported, including changes in the chromosomal structure and microsatellite repeat length.[3,4] To our knowledge, the deletion of mtDNA has not been reported previously, although copy number instability of mtDNA in LCLs has been reported.[5]

Several point mutations near this region cause mitochondrial disorders. For example, the tRNA^{Lys} m.8344A>G mutation causes myoclonic epilepsy associated with ragged-red fibers (MERRF)[6,7] (Figure 2). Therefore, the deletion is likely to have severe functional consequences. This LCL, however, grew normally under standard culture conditions, and fluorescence-activated cell sorter analysis revealed no alterations in the cell cycle (data not shown). Such a mutation may be the source of the phenotypic variability of LCLs,[8] and should be carefully considered when working with LCLs.

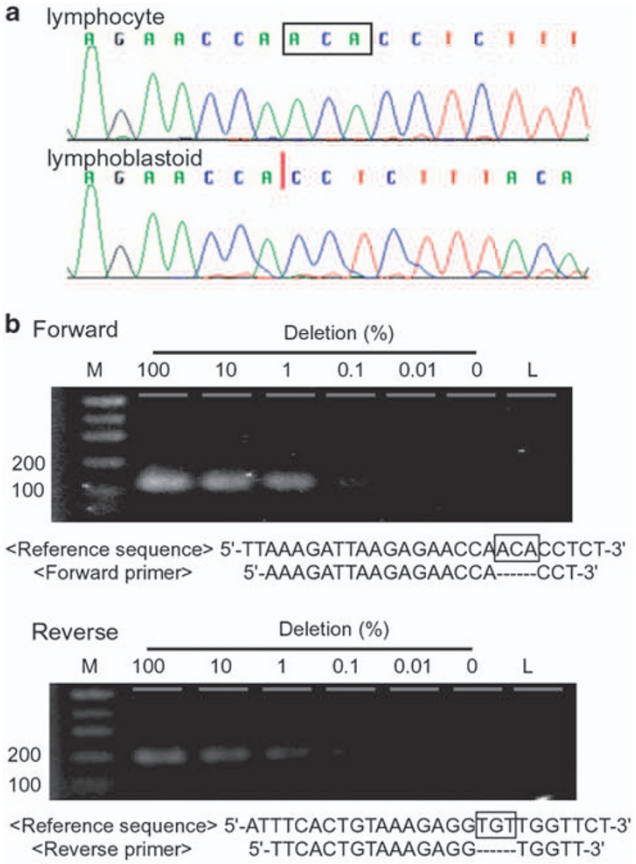

Figure 1 (**a**) A 3-bp deletion in a lymphoblastoid cell line (LCL). Sequencing analysis of mitochondrial DNA around the 3-bp deletion (8348–8350) region in lymphocytes (top) and LCL (bottom) taken from the same individual. The deleted region is shown in the black box in the lymphocyte sequence and as the red line in the LCL sequence. (**b**) The results of allele-specific polymerase chain reaction. We designed allele-specific primer pairs in both forward (5′-AAAGATTAAGAGAACCACCT-3′ and 5′-GTTTAAT ATTTTTAGTTGGGT-3′) and reverse (5′-GTGGAGCAAACCACAGTTTC-3′ and 5′-TTCACTGTAAAGAGGTGG TT-3′) orientations to distinguish the 3-bp deletion shown in the black box. Standard samples were prepared using an LCL with 3-bp deletion (considered a 100% deletion) and another LCL with no deletion (considered a 0% deletion). M, marker (bp); L, lymphocyte sample.

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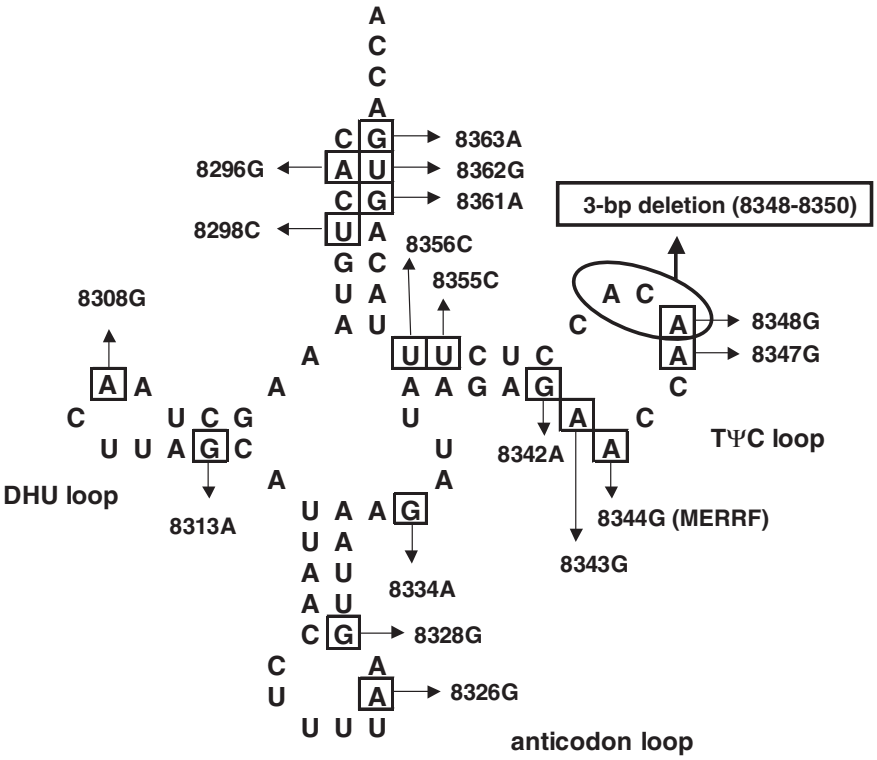

Figure 2 Two-dimensional structure of mitochondrial $tRNA^{Lys}$. The reported pathogenic mutation and polymorphic substitutions according to databases are indicated by boxes.[9–11] The 3-bp deletion is located in the TΨC loop and is indicated by an ellipse.

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