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Letter to the Editor

Mitochondrial DNA mutations may not be frequent in patients with aplastic anemia - *Genet. Mol. Res.* 11 (3): 2130-2137 "Complete sequence analysis of mitochondrial DNA of aplastic anemia patients"

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Dear Editor,

In a recent report, Cui et al. (2012) analyzed the complete sequences of the mitochondrial genome of patients with aplastic anemia (AA). They concluded that (mtDNA) mutations are more frequent in patients with AA. Owing to its high mutation rate, DNA defects may occur at any nucleotides of the 16,569-bp mitochondrial sequence. Mitochondrial dysfunctions have been found to be associated with a wide range of clinical disorders (Wallace, 2010). However, after reading this manuscript, we concluded that the authors' arguments were somewhat compromised and that they presented several statements that were inaccurate and misleading to the readers.

First, Cui et al. (2012) studied 15 patients with AA and collected the oral epithelial cells from these patients as controls to analyze the potential pathogenic mtDNA mutations associated with AA. However, a case-control study is an analytical study, which compares

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individuals who have a specific disease (cases) with a group of individuals without the disease (controls) (Porta, 2008). According to that standard, the selection of the control group should be the bone marrow from healthy individuals. We also suggested that an extended study including a large cohort of subjects is needed to verify the conclusion.

Second, Cui's study designed eight primers to amplify the mitochondrial genome, and subsequently sequenced the PCR products. Considering the size of the PCR products presented in Table 2 Cui et al. (2012), apparently the authors did not take into account the possibility that PCR products greater than 2000 bp would be difficult to sequence because the concentration of DNA that would be analyzed would normally be too low (Pareek et al., 2011). Generally, 24 overlapping fragments using sets of he light-strand and heavy-strand oligonucleotide primers are needed to amplify the mitochondrial genome (Rieder et al., 1998).

Third, Table 1 in Cui's study listed the heteroplasmic mtDNA mutations associated with AA. Nonetheless, a careful check of the candidate "pathogenic" mutations led us to identify various errors in that table: the 14693A>G mutation occurred at position 54 in the T arm of $tRNA^{Glu}$; 10055A>G mutation occurred at position 70 in the acceptor arm of $tRNA^{Gly}$, which disrupts the highly conserved base-pairing (3T-70A) of this tRNA (http://www.mitomap.org/MITOMAP). Apparently, nucleotide alternations in mt-tRNA genes will not affect the amino acid sequence. Moreover, two synonymous mutations: ND1 3834G>A (Leu→Leu) and 4248T>C (Ile→Ile), which do not result in an amino acid change, were wrongly classified as the potential "pathogenic" mutations. Also the 12038A>T mutation in the gene encoding the ND4 subunit is a missense mutation, which replaced the normal codon (AAA) with a stop codon (TAA). In addition, the ND5 13928G>C mutation, which was presented in Figure 1D in the Cui et al.'s paper, was also claimed to be a pathogenic mutation. To check this association, we performed a phylogenetic analysis of this mutation from different species, and the conservation index was then calculated by comparing the human nucleotide variants with those of other vertebrates. As shown in Figure 1, it is obvious that the 13928G>C mutation was not evolutionary conserved and apparently would not have a pathogenic role in the clinical manifestation of AA.

Figure 1. Alignment of the ND5 13928G>C mutation in various species; the arrow indicates the amino acid that is changed at position 531, corresponding to the 13928G>C mutation.

Although the Cui's paper has multiple flaws in sample collection, experimental design and dataset analysis, we also think that mtDNA mutations play an important role in phenotypic manifestation of AA; a careful reassessment of this type of analysis would be warranted to prove this point.

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