



Mitochondrial ND3 G10398A mutation: a biomarker for breast cancer

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ABSTRACT. Mitochondrial DNA mutations have been found to play important roles in carcinogenesis. The most common G10398A mutation, a non-conservative amino acid substitution from Thr to Ala, seems to be involved in the tumorigenesis of breast cancer. Results from studies concerning this mutation remain inconclusive. In the current study, we first took clinical and molecular datasets from case-control studies to determine the association between the G10398A mutation and breast cancer. We further used the Phylotree to determine the haplogroups of this mutation. The frequencies of this mutation in 500 unrelated healthy controls were

also screened. We found that this mutation is very common in the human population, and may be a polymorph.

Key words: Breast cancer; G10398A mutation; Haplogroup

INTRODUCTION

Mitochondria play important roles in cellular energy production, free radical generation, and apoptosis (Verma and Kumar, 2007). Mitochondrial DNA (mtDNA) G10398A polymorphism results in a non-conservative amino acid substitution (Thr to Ala) within the NADH dehydrogenase 3 (ND3) subunit of complex I of the electron transport system. This change is thought to result in increased oxidative stress (van der Walt et al., 2003). This particular polymorph has also been reported to alter both mitochondrial pH and intracellular calcium levels (Kazuno et al., 2006; Kazuno et al., 2008); these alterations have been associated with modulation of ATP production and apoptosis (Bernardi, 1992). Canter et al. (2005) reported that the mtDNA 10398A genotype was associated with an increased risk of breast cancer in African-American women (OR = 1.6, 95%CI = 1.10-2.31, P = 0.013). Moreover, the authors suggested that interaction with unknown genetic and environmental risk factors may cause discrepancies between ethnic groups. On the other hand, Setiawan et al. (2008) reported that this mutation was not associated with breast cancer in African-American women.

It is well established that specific mtDNA polymorphisms create groups of related mtDNA haplotypes or haplogroups (lineages), which are continent specific (Torroni et al., 1996). These lineages are derived from super-cluster L, which are further divided into the subgroups L1, L2, and L3. L3 represents the root of almost all mtDNA diversity outside Africa, differentiating into the major haplogroups, M and N (Watson et al., 1997). Europe, North America, and Australia have very high frequencies of haplogroup N sublineages, whereas in Asia, both M and N sublineages contribute differentially in different regions.

Based on previous observations, in this study, we examined whether the ND3 G10398A mutation contributes to the development of breast cancer. We first carried out a database search for published resources concerning the G10398A mutation. We also analyzed haplogroup distributions and frequencies of the G10398A mutation. Moreover, we performed a mutational screening from 500 unrelated health controls to determine whether G10398A is a neutral polymorphism.

MATERIAL AND METHODS

Database searches

We perform literature searches in Pubmed central and Google scholar by entering the following keywords, "mtDNA, G10398A mutation, breast cancer" to identify published case-control studies investigating the association between the G10398A mutation and breast cancer.

Haplotype/haplogroup assignment

As specific mtDNA polymorphisms create groups of related mtDNA haplotypes or haplogroups, to find haplotype/haplogroup bearing the G10398A polymorphism, we utilized the Phylotree to determine specific haplotypes/haplogroups.

Clinical validation

A total of 500 sporadic unrelated health controls including 250 male and 250 females were recruited in the case-control study. Age of participants ranged between 18 and 80 years of age with a median of 50. The primers for PCR amplification spanning the ND3 region were as follows: Forward: 5'-TCT CCA TCT ATT GAT GAG GGT CT-3'; reverse: 5'- AAT TAG GCT GTG GGT GGT TG-3'. Following PCR amplification, fragments were purified and subsequently analyzed by direct sequencing in an ABI 3700 automatic DNA sequencer using the Big Dye Terminator Cycle sequencing reaction kit (Applied Biosystems, Inc., Foster City, CA, USA, Version 3.1). The sequencing data was compared with the reversed consensus Cambridge sequence (GenBank Accession No. NC_12920) (Andrews et al., 1999).

RESULTS

Relationship between the G10398A mutation and breast cancer

We found 6 studies examining the association between the G10398A mutation and breast cancer (Mims et al., 2006; Bhat et al., 2007; Darvishi et al., 2007; Setiawan et al., 2008; Kulawiec et al., 2009; Czarnecka et al., 2010). Among these reports, some studies claimed a positive association between this mutation and breast cancer, while others claimed that this mutation was not associated with breast cancer.

Phylogenetic analysis for the G10398A mutation

According to the Phylotree, the ND3 G10398A mutation belonged to the human mitochondrial haplogroup B4c1c (Figure 1).

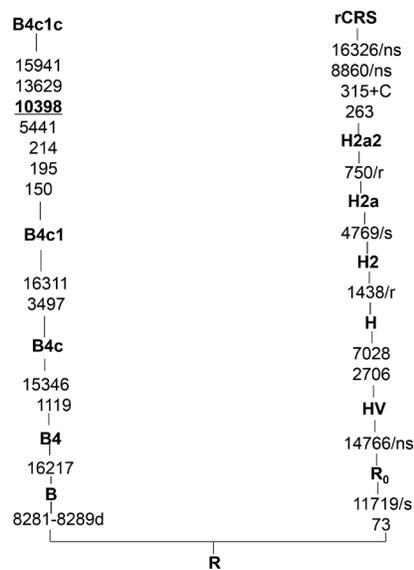


Figure 1. Phylogenetic analysis for the ND3 G10398A mutation.

Screening for the G10398A mutation in 500 unrelated health controls

To determine its relative frequency in the general population, we performed a mutational screening for the G10398A mutation in 500 unrelated health subjects. We found that there were 158 individuals (77 males and 81 females) carrying this mutation, with a rate of 31.6%, suggesting that this mutation is very common in human population (Figure 2).

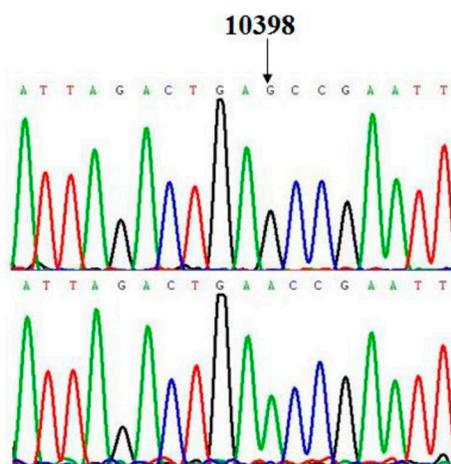


Figure 2. Identification of the ND3 G10398A mutation.

DISCUSSION

In this study, we investigated the possible relationship between the ND3 G10398A mutation and breast cancer. Somatic mtDNA mutations have been reported in various types of cancers, and are associated with the development of cancer (Modica-Napolitano and Singh, 2004; Singh and Kulawiec, 2009). To date, several studies reported an association between the ND3 G10398A mutation and breast cancer. Among them, Canter et al. (2007) described that G10398A polymorphism occurs at a low frequency in the African-Americans, but is prominent among African-American women with aggressive breast cancer. Darvishi et al. (2007) also reported that the G10398A polymorphism increased the risk of breast cancer in the Indian population. In addition, an analysis of 69 polymorphisms in European American women showed that G10398A was associated with increased breast cancer risk (Bai et al., 2007). This mutation is also reported at high frequency in several Asian populations (Kato et al., 2004; Zafarina, 2004). However, the literature contains multiple conflicting reports regarding which nucleotide sequence is associated with cancer. As different populations have variable breast cancer susceptibility associated with this polymorphism, we focused on the possible association between this mutation and breast cancer in the Han Chinese population.

Overproduction of ROS in a cell could lead to many ailments including cancer. There is increasing support for the hypothesis mitochondrial G10398A mutation leads to overproduction of ROS by altering complex I. Tengku et al (2012) suggested that alterations in Complex I of the electron transport chain may cause dysfunction of proteins involved in apoptosis signaling cascades, and thus sensitize normal cells to undergo apoptosis. This allows cancerous cells, which are protected against the induction of cell death, to become the dominant surviving cells.

We found the 10398-nucleotide region in the human mitochondrial genome to be highly polymorphic, which is consistent with published data. The frequency of the ND3 G10398A mutation was found to be 31.6%. In human mitochondrial genetics, haplogroup N is derived from the ancestral L3 haplotype that represents the “Out of Africa” migration. It is the ancestral haplogroup to almost all European and Oceanian haplogroups in addition to many Asian and Amerindian ones. Superhaplogroup N can be divided into smaller haplogroups such as R, A, I, S, W, X and Y (Kong et al., 2011). Haplogroup N is found in all parts of the world, but has low frequencies in Sub-Saharan Africa, and can be defined by the mutations at position 8701; 9540; 10398; 10873 and 15301 (van Oven and Kayser, 2009).

In conclusion, the haplogroup B4c1c specific ND3 G10398A mutation was found to be a very common genetic marker in the human population, and may not be associated with breast cancer.

Conflicts of interest

The authors declare no conflict of interest.

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