Letter to the Editor

Mitochondria DNA 4 977 bp Common Deletion in Peripheral Whole Blood from Healthy Donors*

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To investigate the distribution of mitochondria DNA 4 977 bp deletion, a common deletion (CD), in normal populations of Chinese, human peripheral blood samples from sixty healthy donors were collected, and levels of the CD in genomic DNA from the samples were detected using real-time PCR. The results showed that the CD was found in 27 health donors, with its positive rate being 45% (27/60). The CD ratio was between 0 and 0.000308%, and not affected by age and gender in sixty healthy donors. Our studies indicate that the CD ratio is low, and do not show the age-dependent accumulation and any gender difference in peripheral whole blood from the normal Chinese population.

The mitochondria DNA (mtDNA) with 4 977 deletion mutation from base-pairs 8 470 to 13 446 in human mtDNA map of Anderson is a common type of mtDNA deletion mutation, referred to as the common deletion (CD)[1]. The CD removes the genes or parts of the genes encoding for ATPase 8 and 6, COXIII, ND3, ND4 and ND4L and ND5 of the mtDNA[2], resulting in an impairment of the mitochondrial oxidative phosphorylation[3], which has been shown in various tissues from aging humans, mitochondrial myopathy and cancer[4-5]. And it has been reported that the CD is accumulated in different postmitotic tissues in an age-dependent manner[4]. However, only few experiments have evaluated the basal level of the CD in human peripheral blood from the normal population till now. Meissner et al.[6] detected the CD in peripheral whole blood samples from ten healthy people aged from 20 to 71 by real-time PCR assay, and found that the amount of the CD detected in each sample did not exhibit any difference or age-dependent accumulation. The similar result was obtained by Mohamed et al.[7] and our previous study[8]. The former studied genomic DNA samples of peripheral whole-blood from 10 healthy donors (5 individuals aged from 19 to 22 years, 5 aged from 57 to 61 years), while the latter investigated mtDNA samples of peripheral whole-blood from 27 healthy individuals using real-time PCR[7-8]. However, the sample size was too small in these two studies, and the distribution of the CD with enlarged sample size of healthy individuals has remained unclear. Therefore, we detected the CD in peripheral whole-blood samples from sixty healthy subjects using real-time PCR in the present study, and further analyzed the basal level of the CD in healthy individuals with enlarged sample size. This will provide some basal data of the CD in order to establish a new ionizing radiation (IR) biodosimeter by the CD measurement.

First, a total of 60 healthy volunteer individuals at the Henan Institute of Occupational Medicine (HIOM) were recruited to study the distribution of mtDNA CD in a normal population. The eligibility of the 60 healthy adults (33 males and 27 females) aged 20-80 years, was evaluated using questionnaires and regular medical procedures. This work was conducted at the HIOM. The aim of the study was explained to each subject, and all experiments of the study were approved by the Ethics Committee of HIOM. After written informed consent was obtained, approximately 1 mL peripheral blood sample was collected from each subject by venipuncture into vacutainers containing EDTA. The total DNAs (including mitochondrial and nuclear DNA) from 250 μL blood samples were extracted using the AxyPrep Whole Blood Genomic DNA Miniprep Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and diluted in 100 μL TE buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 8.0). Total DNA's quality and quantity were determined by

doi: 10.3967/ bes2013.035

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separating aliquots of the DNA in 1.0% agarose gels with ethidium bromide and the intensity of signals to known amounts of DL 2 000 DNA Marker (Takara, Dalian, China) was compared using a Syngene Bioimaging System (Synoptics Ltd., Cambridge, UK). The remaining DNA sample was stored at -20 °C.

PCR primers for the quantitative PCR (Q-PCR) were designed according to MITOMAP Human Cambridge Sequence data (www.mitomap.org) to detect the CD from the health donors. A 151-bp region of the 12S rRNA gene in the heavy strand was used to represent the total amount of mtDNA since this region is relatively conserved. Another 151-bp region spanning the deletion junction was also used to represent CD. The forward primers (12SrRNA: 5'-AAATCCACCTTCGACCCTTAAGT-3'; CD: 5'-ACCCCCAT ACTCCTTACACTATTCCT-3'), reverse primers (12S rRNA: 5'-AACCCTGATGAAGGCTACAAAGTAA-3'; CD: 5'-CGGTTTCGATGATGTGGTCTTT-3') and TaqMan hybridization probes (12S rRNA: 5'-FCCATTTCTT GCCACCTCATGGGCTACP-3'; CD: 5'-FCCACCTACCTCC CTCACCATTGGCAP-3') were synthesized by GeneCore Bio Technologies Co. Ltd (Shanghai, China). Both plasmids containing the breakpoint and the 12S rRNA region were previously constructed by our laboratory[8]. Dose-dependent plasmid-constructed 12S rRNA and CD standards were used in each run of real-time PCR. All TaqMan reactions were carried out in 96-well plates on an ABI 7 500 Real-Time PCR instrument (Applied Biosystems, CA, USA) using the real-time PCR Master Mix kit from Takara (Dalian, China). Each PCR reaction was carried out in total volume of 20 μL containing 100 ng total DNA template, 200 nmol/L primer, and 200 nmol/L TaqMan probe. After an initial denaturation step at 95 °C for 20 s, 40 PCR cycles of 5 s at 95 °C and 34 s at 60 °C were performed. Real-time PCR of all samples and standards were performed in triplicate. The data from a PCR run were rejected if the correlation coefficient of the standard curve was less than 0.98. All statistical analyses were conducted using SPSS, version 15.0 (SPSS, Chicago, USA). Data were presented as the mean±SD. Differences in mtDNA and CD level were analyzed by nonparametric test (Mann-Whitney *U* test) and independent samples *t* test. *P* values <0.05 are considered statistically significant. All reported *P* values are two sided.

To determine level of the CD, the copy number of CD and total mtDNA in human peripheral blood samples from sixty healthy donors was detected by real-time PCR, and the CD rates were calculated as the copy numbers of CD molecules per total mtDNA molecules ($CD/mtDNA^{total}$). The results showed that the CD was observed in genomic DNA samples of 27 health donors, with its positive rate being 45% (27/60). But it was low when compared with our previous study, in which the 70.37% (19/27) of the CD positive rate was found in mtDNA samples of peripheral whole-blood from 27 healthy donors aged from 17 to 44 years by real-time PCR[8]. This might be interpreted with the difference in the used samples, which was the mtDNA from whole-blood cells in the previous study.

The distribution of the CD ratio in sixty healthy donors is shown in Figure 1. The CD ratio was very low and between 0 and 0.000308%. According to their age and gender, sixty healthy donors were divided into two groups (Table 1). The mean of the CD copy number and total mtDNA copy number in the ≤50 year age group was higher than that in the >50 year age group, but no statistically significant difference was observed between the two groups (U=338.500, P=0.071; t=1.581, P=0.119). Although the mean of the CD copy number and total mtDNA copy number in the male group was higher than that in the female group, the difference was not statistically significant between the two groups either (U=332.000, P=0.065; U=353.000, P=0.169). However, the mean of the CD ratio in the >50 year and the male groups were higher than that in the ≤50 year and the female groups, but the differences between the groups still were not obvious (age: χ^2=4.052, P=0.542; gender: U=355.000, P=0.141; Table 1), namely neither age-dependent accumulation[6-8] nor any gender difference was observed. And the mean of the CD ratio in this study was very low, about 0.000051%±0.000078 (Table 1), but it was higher than that in the report by Mohamed et al. (0.000036%±0.000056)[7]. This may have some connection with our data from the normal Chinese population.

In addition, the CD can be induced by IR, and showed a dose-dependent increase with some dose range. Recently, it has been demonstrated that the relative amount of the CD in human peripheral blood exposed to ^{60}Co gamma ray showed certain dose-dependent increase between 0 and 8 Gy at 2 h after exposure using real-time PCR in relative quantification[9]. Our study showed that the mean of the amount of the CD ratio also exhibited a dose-dependent increase in the dose range from 1 to 5 Gy in peripheral whole-blood from six healthy donors at 2 h after irradiation by TaqMan real-time

Table 1. CD and Total mtDNA Levels, and CD Ratio in Peripheral Whole-blood from Healthy Donors

Groups	Cases	CD Copy Number	mtDNA Copy Number	Ratio of CD (%)
Age				
≤50 year	30	6.58±8.78	10.00 e+006±3.60 e+006	0.000053±0.000064
>50 year	30	5.09±6.74	6.43 e+006±2.02 e+006	0.000075±0.000092
Gender				
Male	33	6.13±8.48	8.17 e+006±4.52 e+006	0.000061±0.000085
Female	27	2.63±4.75	6.47 e+006±2.29 e+006	0.000040±0.000070
Total	60	4.53±7.19	7.46 e+006±3.75 e+006	0.000051±0.000078

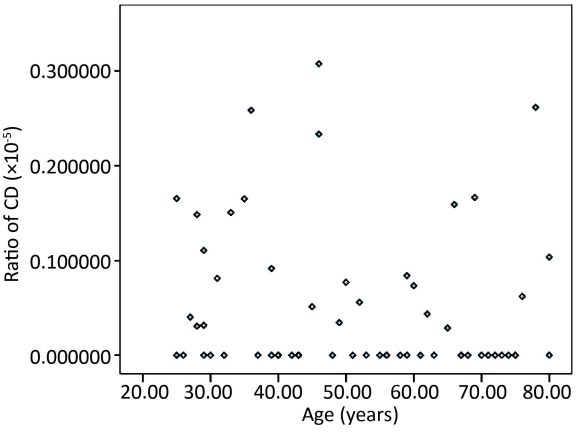

Figure 1. Distribution of the CD ratio in peripheral whole-blood from sixty healthy donors. The CD ratio from every sample is shown in correlation to the age of the individual.

PCR, and the mean of the amount of the CD ratio was between 0.0186%±0.0037 and 0.0386%±0.0193 (data not shown). In irradiated human peripheral lymphocytes from acute lymphoblastic leukemia patients, average 0.53 fold CD levels were detected 24 h after 4.5 Gy total body irradiation (TBI) when compared with their basal levels, and 9 Gy TBI produced a greater response of the CD levels than 4.5 Gy using real-time PCR method[10]. These results indicate that the CD levels can be induced by IR in human blood cells *in vitro* and *in vivo*, and exhibit certain dose-dependent correlation. So it might be an ideal biomarker related to IR.

In summary, the basal level of the CD was very low and none of the peripheral whole-blood exhibited an age-dependent accumulation of the deletion and any difference in both genders, which can meet the basic needs for constructing IR boidosimeter. Furthermore, it could be induced by IR, so it might be a candidate of molecular biomarker associated with IR for constructing a new biodosimeter.

*This work was supported by grants from the building project for the National Key Clinical Special Department of China (No. 2011-17), the Medical Science and Technology Foundation of Henan Province (No. 201204123) and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

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Received: April 26, 2013;

Accepted: June May 3, 2013

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