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SUSCEPTIBILITY ON HEARING OF WORKERS FOR NOISE EXPOSURE AND MITOCHONDRIAL DNA 7.4 KB DELETION

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ABSTRACT
Purpose: To explore the association between susceptibility of hearing for noise exposure and mitochondrial DNA (mtDNA) deletion. **Subjects and Methods**: The DNA was collected from white blood cells of 41 workers. Twenty one of them were susceptibility on hearing to long-term noise exposure and the other twenty were not susceptibility. An advanced PCR technique was applied to observe the 7.4kb mtDNA deletion. **Results**: Four kinds of deletion were found from 3.1kb to 7.4kb in these workers. The 7.4kb deletion frequency in susceptibility group (10/21) was significantly higher than that in control group (3/20), OR=5, P<0.05. **Conclusion**: This result suggests that the 7.4kb mtDNA deletion is one of possible risk factors for individual susceptibility on hearing to noise exposure. It is long time to know the stories about a few 'glass ear' and 'iron ear' in noise exposure population. It still dose not know what substances in human body induce different hearing damage by same dosage of noise exposure. A new hypothesis was created to say the deletion of mitochondrial DNA (mtDNA) might be one of the reasons for susceptibility of hearing on noise exposure. In this paper, 7.4 kb deletion of mtDNA was observed in typical susceptibility workers and non-susceptibility workers from a few textile and mechanical factories.

1 - SUBJECT AND METHODS

The subjects were defined as workers who were worked in noisy environment equal or more than one year and had personal records of noise exposure data and health examination information in their factories. More than one hundred of them were included in the project either possible susceptibility or non-susceptibility by auditory threshold distribution model with hearing and noise exposure data from their personal record. A health examination and questionnaire were taken to all of the subjects. Audiometric thresholds were measured by NTB-40 audiometer from 125 Hz to 8 kHz at both ears in sound barricaded room after leaving noisy environment more than 16 hours. Sound pressure level of noise was measured by ND-2 or HS 5670 sound level meters in working position at ear level. The questionnaire table included the personal general information, occupational history, personal and family diseases history. By auditory threshold distribution model, 22 of susceptibility cases and 22 non-susceptibility cases (control group) were found from above subjects. Blood samples were collected from these cases with EDTA-Na_2 pretreatment and 6 ml for each one. All samples were kept in -20°C icebox. DNA was prepared from blood sample by routine phenol-chloride extracted method.
5' primer (3108-3127) and 3' primer (3731-3712) were designed to amplify D-loop of mtDNA. 5' primer (8150-8166) and 3' primer (16159-16142) were designed to amplify 7.4 kb mtDNA deletion.

10 ×PCR buffer (including 15mM $MgCl_2$)	2.5 μl
4 ×dNTP (2.5mM)	2.5 μl
5'primer (12.5 μM)	1 μl
3'primer (12.5 μM)	1 μl
Taq DNA polymerase	1 U
DH_2O	15 μl
DNA template (50ng/ μl)	3 μl

Table 1: Reaction system of PCR for detecting 7.4 kb deleted mtDNA.

Table 1 showed the reaction system of PCR with Omn-E DNA thermal cycler. The schedule of PCR was 94°C denature for 3 minutes, 94°C denature for 50 seconds, 51°C (54°C) anneal for 1 minute, 72°C extend for 1 minute with 35 thermal cycles, 72°C extend for 10 minutes at last of the schedule. Success of amplification was determined by electrophoresing 10ml of the PCR product on a 1.5% agarose gel.

2 - RESULTS

All subjects did not wear earplugs or earmuff during their noisy working histories. The table 2 showed the distribution of sex, age and noise exposure between the two groups on balance without significant. It was a significant peak of hearing threshold about $3 - 8$ kHz in susceptibility workers with feature of typical noise induced hearing damage. The hearing thresholds of susceptibility workers were significantly higher than those of control workers from 125 Hz to 8 kHz. The hearing thresholds had near levels same trends on both ears.

Parameter	Susceptibility Group (n=21)	Control Group (n=20)
Male/Female	7 / 14	5 / 15
Age	32.2 ± 4.5	34.0 ±10.5
SPL(dB(A))	91.2 ± 8.2	93.3 ± 6.0
CNE(dB(A))	100.0 ± 9.9	101.3 ± 8.2
Working years	12.0 ± 4.9	10.6 ± 8.8

Table 2: Comparison of sex, age and noise exposure for susceptibility and control groups.

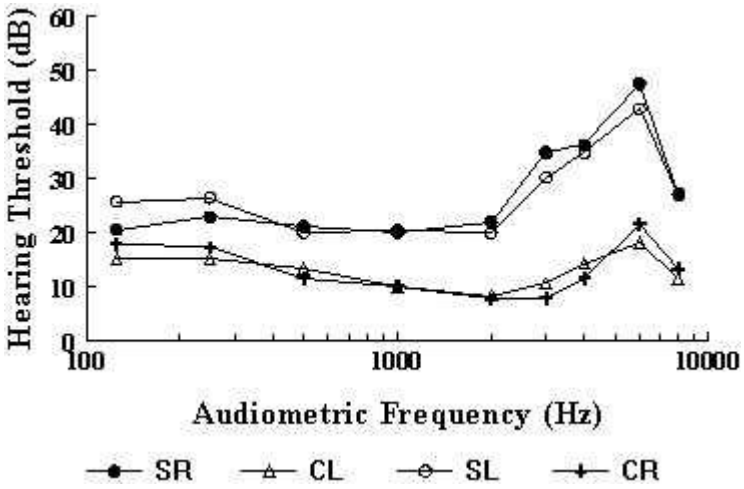

Figure 1: Comparison of hearing thresholds for left ear of susceptibility workers (SL), right ears (SR) and left ear of control workers (CL), right ears (CR).

Figure 3 showed results of 7.4 kb detection of mtDNA with primer (8150-8166/16159-16142) by 8 kb distance. Four DNA pieces were collected with 4.90 kb, 1.37 kb, 831 bp and 594 bp. By these results, four lengths of mtDNA deletion could be calculated as 3.10 kb, 7.63 kb, 7.17 kb and 7.41 kb. The distribution of mtDNA deletion was summarized in table 3 with higher deletion ration (10/21) in susceptibility workers and lower (3/20) in control workers, OR=5, $P<0.05$. This result suggested that the 7.4 kb deletion of mtDNA might increase risk for workers with susceptibility on hearing for noise exposure.

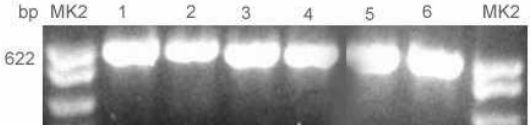

Figure 2: PCR product of total mitochondrial DNA, 1-3: control cases, 4-6: susceptibility cases, MK2: PBR322 DNA/Mspl Markers.

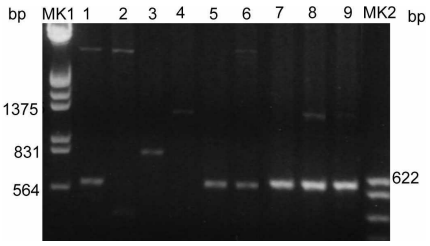

Figure 3: 1-9: PCR Product of 7.4Kb deleted mtDNA, MK1: lDNA/HindIII+EcoRI Markers, MK2: MK1:PBR322 DNA/Mspl Markers.

Group	mtDNA 7.4kb deletion	
	+	-
Susceptibility	10	11
Control	3	17

Table 3: Relation between positive reaction of mtDNA 7.4kb deletion and hearing susceptibility in noise exposure workers (X^2_{M-H}= 4.91, df=1, P<0.05).

3 - CONCLUSION

Genetic issue about noise induced hearing loss is a new research area in occupational and otological sciences. The recent data (1998) show the extreme hearing thresholds of a noise exposure population can arrive about 100dB after adjusted age, sex, noise exposure level and duration [9]. It means the individual susceptibility of hearing to noise exposure is very significant issue. The potential associated substances in human body might be some enzymes or receptors or some antagonists or other substances, which associate with hair cell surviving during noise exposure. It is known that peptide sequence is decided by genes. By this reason, looking for mutation of DNA is a reasonable strategy to find substances which are associated with noise induced hearing damage.

Hyde (1995) used chickens to observe the possible mechanism for hearing damage. The results found that the hair cells of chicken had light damage by sound exposure (120 dB, 120Hz and 14 hr.) or chlorimyocine treatment. But the hair cells of chicken were seriously damaged by chlorimyocine pre-treatment and sound exposure (120 dB, 120Hz and 14 hr.) [5]. It is known that the chlorimyocine can stop process of protein synthesize in mitochondria to reduce oxidativephosphorylative ability of inner ear. After chlorimyocine treatment, the hair cell can not get more energy by oxidativephosphorylation process. The hair cell will under condition of lacking more energy supply. Lack of energy supply can induce damage of hair cells and hearing loss.

In publications, the deletion of mtDNA associate with some later-onset diseases [6] which are very like the feature of noise induced hearing damage. Tree kinds of mtDNA deletion had been found of 5kb, 7.4kb and 10.4kb in these diseases. In this paper, 7.4 kb deletion of mtDNA was selected as a candidate mutation.

It was found a higher frequency of 7.4 kb deletion of mtDNA in susceptibility cases than that in non-susceptibility cases in this paper. The crude result suggests that the deletion of mtDNA might be one of genes for noise induce hearing damage. It might be associated with the deletion of mtDNA which impair the protein synthesis of the mitochondrial subunits of the respiratory chain complexes.

It is known that noise pre-treatment can reduce noise induced hearing loss [3]. One possible explain is to say the result might be caused by inducing more proteins of the mitochondrial subunits of the respiratory chain complexes during noise pre-treatment. More proteins of the mitochondrial subunits of the respiratory chain complexes can supply more ATP in hair cell to reduce damage when it exposed to noise.

This paper just uses a small case-control population to observe the relation between mtDNA 7.4 kb deletion and noise induced hearing damage. More subjects and more kinds of mutation need to be included in future investigation to establish an overview of mechanism for noise induced hearing damage.

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