Role of UCP1 and UCP2 gene polymorphisms in Presbycusis

Manche Santoshi Kumari*†, Jangala Madhavi*†, Koralla Raja Meganadh*, and Akka Jyothy†

*MAA Research Foundation, Hyderabad, Telangana State, India
Institute of Genetics and Hospital for Genetic Diseases, OU, Telangana State, India

Introduction

Presbycusis (adult onset hearing loss) is a multifactorial non-syndromic disorder caused by an oxidative stress and mitochondrial dysfunction. The uncoupling proteins (UCPs) are members of mitochondrial anion carrier protein family which are involved in controlling the level of respiration coupling. Polymorphisms in UCP genes especially UCP1 and UCP2 are associated with age related hearing loss.

Objective

To investigate the association of the uncoupling protein 1 (UCP1 A-3826G) and uncoupling protein 2 (UCP2 G−866A) polymorphism with presbycusis in South Indian population.

Subjects and Methods

Subjects

A total of 220 cases from 2011-2014 who were visiting MAA ENT hospitals and confirmed of hearing loss were considered as the study subjects. The patients whose age was equal or greater than 40 years with no vestibular or any history of otological surgery, sensorineural form of hearing loss (bilateral as well as symmetrical), familial history and complications such as diabetes, hypertension and hypothyroidism were included in the study. The study was approved by Institutional ethics committee. Those without any hearing loss and disease considered as healthy controls. After informed written consent, 2 ml of blood was collected groups were kept in EDTA vials for analysis.

Genotyping

Genomic DNA was isolated from whole blood samples by salting out method of Lahiri et al., 1991. PCR RFLP method was used to determine UCP1 and UCP2 polymorphisms in the isolated DNA. For the UCP1 A(-3826)G polymorphism, 5'-CTTGGGTAGTGACAAAGTAT-3' forward and 5'-CCAAAGGGTCAGATTTCTAC-3' reverse primers were used and the PCR product was subjected to *BclI* restriction digestion (Cassard-Doulcier et al., 1996). In UCP2 G(−866)A polymorphism was amplified by 5'- CACGCTGCTTCTGCCAGGAC-3' as forward primer and 5'-AGGCGTCAGGAGATGGACCG-3'as reverse primer (Sesti et al., 2003). PCR products were digested by *MluI* restriction enzyme.

Statistical Analysis

Chi-square test and binary logistic regression analysis was used for determining the association of UCP1 and UCP2 genotypes with presbycusis by using the Statistical Package for Social Sciences, PASW STATISTICS 18.0 software (SPSS Inc., Chicago, IL,USA).

Table 1.Distribution of demographic, audiological and co-morbidities in the study subjects

Parameters	Cases (n=220)	Controls (n=270)	P-value†
Gender †			
Male	127(57.7)	160(59.3)	0.73
Female	93(42.3)	110(40.7)	
Age at visit(years)††	63.7±9.98	62.9±9.86	0.396
Age of onset(years)††	60.2±10.38	60.9±10.07	0.885
Degree of hearing loss(Decibels-dB)			
<40 dB	12(5.5)	270(100.0)	NA
>40dB	208(94.5)	0(0.0)	
Co- morbidities†			
Diabetes	48(21.8)	18(6.7)	0.001***
Hypertension	30(13.6)	33(12.2)	0.642
Hypothyroidism	3(1.4)	6(2.2)	0.481

a. †-χ^2- test; ††- Independent 't' test
b. NA-Not applicable
*c. Level of significance of odds ratio: *p-value<0.05,**p-value<0.01, ***p-value<0.001*

Figure 1 . *BclI* of digest of 470 bp fragment containing polymorphism at UCP1 A(-3826)G

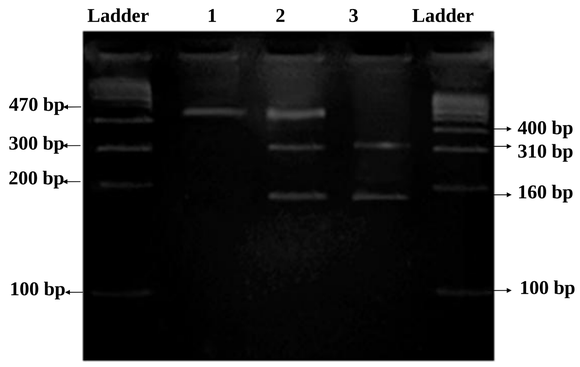

Figure 1 . *MluI* of digest of 363 bp fragment containing polymorphism at UCP21 G(-866)A

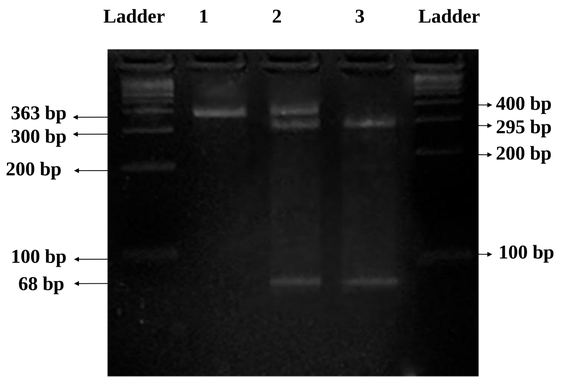

Table 2 .Association of UCP1 A(-3826)G polymorphism

SNP A(-3826)G	Cases(%)	Controls(%)	OR(95% CI)††	P-value†
Genotypes				
AA	45(20.5)	48(17.8)	1.00	0.545
AG	97(44.1)	114(42.2)	0.86(0.51-1.46)	
GG	78(35.5)	108(40.0)	0.79(0.46-1.35)	
Alleles				
A	187(42.5)	210(38.9)	1.00	0.252
G	253(57.5)	330(61.1)	0.86(0.67-1.11)	

a.†- Chi-square 't' test
b. ††- Binary logistic regression analysis

Table 3. Association of UCP2 G(−866)A polymorphism

SNP G(−866)A	Cases(%)	Controls(%)	OR(95% CI)††	P-value†
Genotypes				
GG	49(22.3)	156(57.8)	1.00	<0.001
GA	129(58.6)	93(34.4)	4.42(2.91-6.72)***	
AA	42(19.1)	21(7.8)	6.30(3.41-11.66)***	
Alleles				
G	227(51.6)	405(75.0)	1.00	<0.001
A	213(48.4)	135(25.0)	2.82(2.15-3.69)***	

a.†- Chi-square 't' test
b. ††- Binary logistic regression analysis
*c. Level of significance of odds ratio: *p-value<0.05,**p-value<0.01, ***p-value<0.001*

Results

- Male preponderance is seen in study subjects of presbycusis with mean age of onset is at 60.2±10.38 years.
- UCP1 A(-3826)G genotypes and allelic frequencies did not exhibit any significant association with the presbycusis patients and control subjects.
- UCP2 G(−866)A genotypes GA and AA showed a significant difference between presbycusis and controls. The A allele carriers of UCP2 in the presbycusis patients had significant risk when compared with the GG genotypes.

Conclusion

Mitochondrial uncoupling proteins UCP2 at –866 G/A showed polymorphism which had an influence in the risk of presbycusis onset. Further, the association of AG and AA genotypes of UCP2 with presbycusis indicates a damage caused in the neuron by oxidative process and thermal signaling modulation of inner ear leads to hearing loss.

References

1. Lahiri DK, Nurnberger JI Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies.Nucleic Acids Res. 1991 Oct 11;19(19):5444.
2. Cassard-Doulcier AM, Bouillaud F et al. The Bcl I polymorphism of the human uncoupling protein (ucp) gene is due to a point mutation in the 5'-flanking region. Int J Obes Relat Metab Disord. 1996 Mar;20(3):278-9.
3. Sesti G, Cardellini M, Marini MA et al. A common polymorphism in the promoter of UCP2 contributes to the variation in insulin secretion in glucose-tolerant subjects. Diabetes. 2003 May;52(5):1280-3.