Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.5, No.22, 2015

Molecular Genetic Study of PTC Tasting in Basra Population/Iraq

Faizah A. W. Ahmed[1] Asaad Y. Ayied[2] Hasna A. Mohaus[3]
1. Biology Department, College of Education for pure Sciences, Basra University, Basra, Iraq
2. Animal Resources Department, College of Agriculture, University of Basra, Basra, Iraq
3. College of Dentistry, Missan University, Missan, Iraq

Abstract
The present study was conducting during 2012-2014. Sequencing of *TAS2R38* (P49A) PTC gene 145 C/G) rs713598) among 59 healthy individuals from both sexes and age ranged 30-70 year was studied. Results showed a present of three genotypes included CC, CG and GG. The first two genotypes were tasters while the third was not taster. The frequency of C and G alleles were 0.55, 0.45 among studied individuals. Genotypic frequencies showed a significant access frequency of heterozygous genotype CG as 0.76 which may implies a selective heterozygous advantage among this region of Iraqi population. A significant differences in genotypic distributions with high incidence of GG genotype (non- taster) among people was recorded.
Keywords: PTC; taste; genetic; polymorphism

1- Introduction

Bitter taste is detected by a set of 25 taste 2 receptors (TAS2R) (Meyerhof et al. 2010), and individual differences in the ability to taste substances like phenylthiocarbamide (PTC) have been known since a long time (Fox 1932). Single nucleotide polymorphisms (SNPs) in the TAS2R38 gene have been identified as the key determinants of this capability, as well as for that of tasting the related compound 6-n-propylthiouracil (PROP) (Bufe et al. 2005; Kim et al. 2003; Duffy et al. 2004).

Study traits genetically help us to understand the human dynamic, as traits have different frequencies in different populations that has been used to evaluate and analyze evolution forces as well as taxonomy of human race (Padmavathi, 2013). To realize human diversity, many genetic polymorphisms indicators were used. Those indicators provide important information about mutation, selection, migration and study the correlation with some diseases occur at different places around the world (Wooding *et al*, 2012).

Food play an important rules in development of human being through its impact on human behavior to get its food or eat some types of food (Oliveira, 2012). Therefore, many studies tend towards nutrigenomics (Fogg-Johnson and Kaput, 2003; Kaput and Morine, 2012). Eating vegetables still form the most important healthy nutrition (Bazzan *et al*, 2013). Although there are many factors effect human food consumption, taste is the factor of highest effect dealing with this subject (Turner-McGrievy *et al*, 2013).

Due to the important role of tasting a special science was initiated (Gastronomics) to cover different details on this role through studying the impact of tasting on individual food consumption behavior and individual variation on molecular bases and their effects on the way of consumption and using food. The objectives of this study was to determine Basra population nature through studying genetic variation of the TAS2R38 (P49A) gene responsible for the ability to taste PTC.

2. Materials and Methods

The molecular study included DNA extraction and amplification by using PCR, electrophoreses and gene sequencing.

2. 1. DNA extraction from buckle swab:

DNA was extracted from epithelial cells of 59 individuals from different area of Basra province by using cotton swabs from the mouths. DNA extraction and genotyping were done at the Molecular Oncology Unit Laboratories of Guy's and St Thomas's Hospital /London /UK.

2. 2. Amplifying DNA by PCR and DNA sequencing

A copy of rs713598 which consist of 221 bp of *TAS2R38* gene primer (table, 1) was amplified by using PCR technique (table, 2). DNA sequencing ***performed at*** the Molecular Oncology Unit Laboratories of Guy's and St Thomas's Hospital /London /UK.

Table (1) TAS2R38 (P49A) primer

Primer		Primer sequences	Length	Tm
Forward	F	CCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCGG	46	72.17
Reverse	R	AGGTTGGCTTGGTTTGCAATCATC	24	84.80

Tm: Melting temperature

Table (2) PCR program to amplified *TAS2R38* (P49A) gene

Stage number	Steps	Temperature°	Time	No. cycles
1	Denaturation 1	94 °C	3 min	One
2	Denaturation 2	94 °C	30 sec.	35
3	Annealing	64 °C	45 sec.	
4	Extension	72°C	45 sec.	
5	Extension 2	72°C	5 min	one

2. 3. Statistical Analysis:

Results homogeneity have been tested by $\chi 2$ test by using Genepop program. The program also used to measure some genetic parameters of the studied population. Mutation analyzed by Mutation Surveyor Software V. 5. 2.

3. Results and Discussion

3. 1. Gene sequence TAS2R38 P49A

The genetic sequence was determined for the PCR product to DNA area consisting of 221bp in the rs713598 region of the gene TAS2R38 (photo, 1) which contains the first SNP gene TAS2R38 P49A. Molecular study has shown the existence of the base G or C site 145 c/g which coded to the amino acid No. 49 to be Proline if there is the base C. And that indicates the presence of genotype of tasters in its pure form CC or CG hybrid format. Figures (1, 2 and 3) showed the genotypes GG, CC and CG respectively.

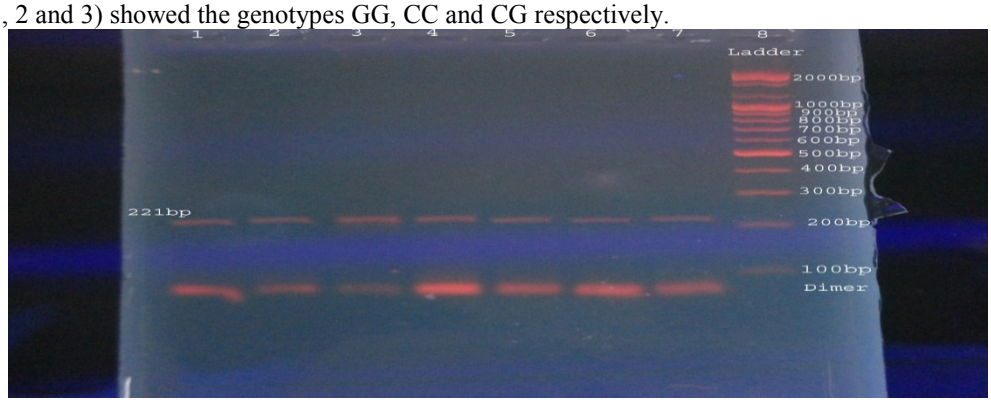


Photo (1). Electrophoresis of PCR product of *TAS2R38* gene band 221bp

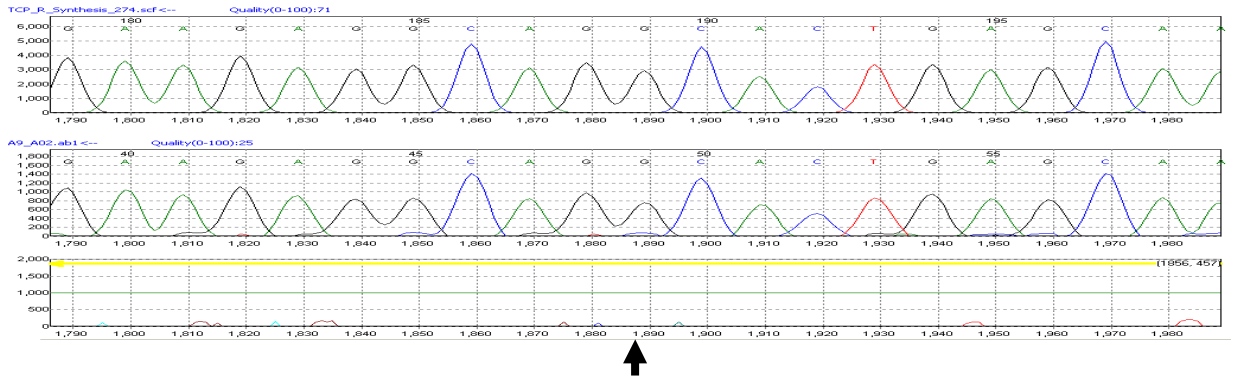
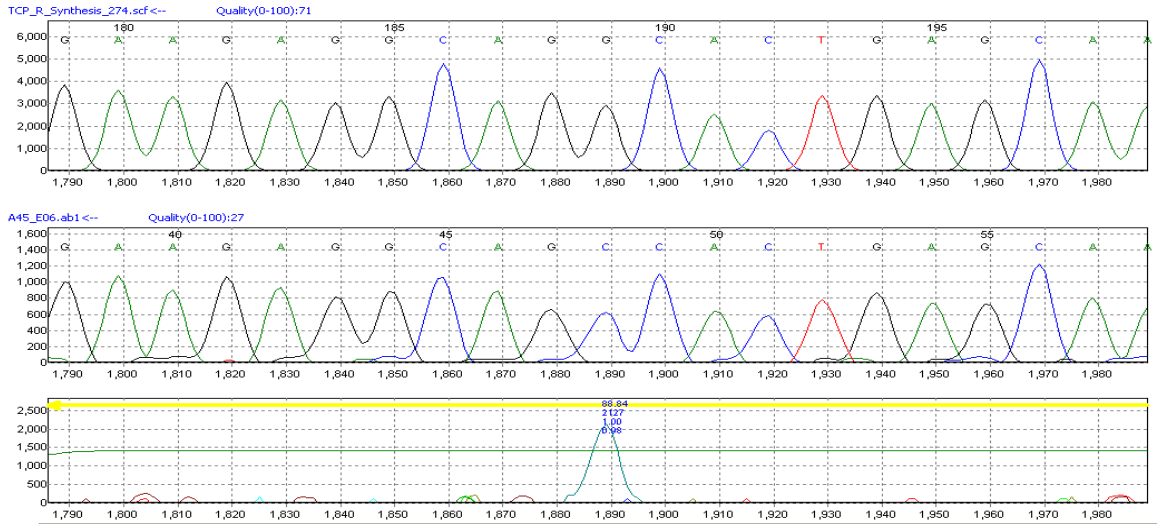
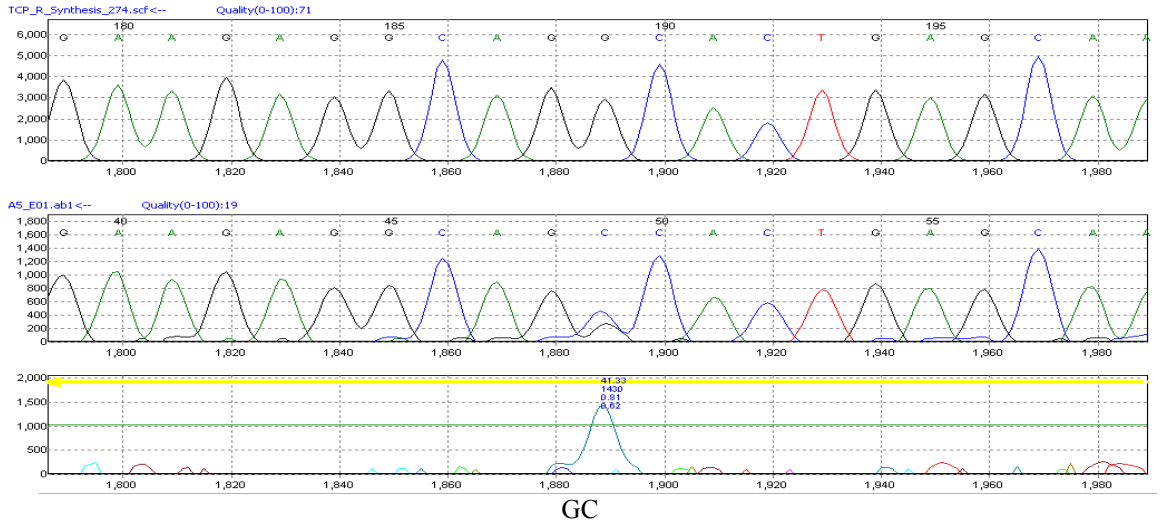


Fig. (1). Pure non-taster genotype (GG, SNP 145 G/G) nucleotide base G of Alanine acid code



C

Fig. (2). Pure taster genotype (CC, SNP 145 C/C) nucleotide base C of Proline acid code



GC

Fig. (3). Pure non-taster genotype (GC, SNP 145 C/G) nucleotide C for Proline acid and base G of Alanine acid code

3. 2. Allele Frequency of *TAS2R38* (P49A):

Table (3) and figure (4) showed the frequency of G and C alleles of studied sample. Frequency of C exceeded that of G (0.55 and 0.45 respectively). Frequency of genotype GC was higher (0.76) than those of CC and GG genotypes (0.17 and 0.07 respectively). When comparing observed and expected number of genotypes, statistical analysis revealed that the population under unbalanced Hardy-Weinberg equilibrium as there is great value of heterozygote (table, 4).

Table (3). Allele and genotypes frequencies of PTC *TAS2R38* gene of Basra population

Genotype	Genotype frequency	Allele frequency	
		G	C
CC taster	0.17	0.45	0.55
GC taster	0.76		
GG non-taster	0.07		

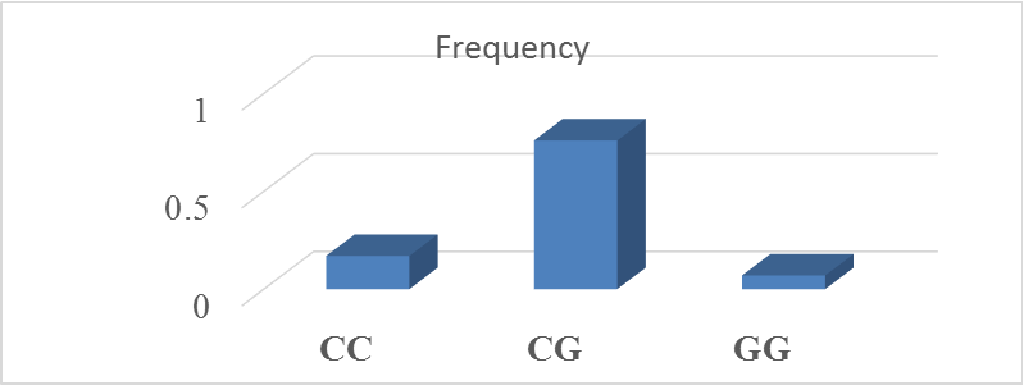


Fig. (4). Genotype frequency of *TAS2R38* (P49A) of Basra population

Table (5). Observed and expected number of *TAS2R38* (P49A) genotypes

Genotypes	Observed number	Expected number	Chi Square ($\chi 2$)
CC (PAV/PAV)	10	17.85	3.40
CG (PAV/AVI)	45	29.20	8.54
GG (AVI/AVI)	4	11.95	5.29
TOTAL	59	59	17.22**

**** There are significant differences at 1%. Expected number=Hardy genotype frequency x total number**

It is clear from these results that the gene *TAS2R38* (P49A) recorded high frequencies from taster of the genotypes CC and CG in comparison with the recessive non-tasted individuals. These results are in consistent with international studies, as in Malaysia (Ooi et al, 2010), Turkey (Ergun and Askoy, 2013) and USA (Schembre *et al*, 2013). C allele also recorded highest values in comparison with G allele. Behrens et al (2013) recorded similar result (C frequency=0.54). In contrary, Wooding *et al* (2004) and Kim *et al* (2003) estimated G frequency as 0.55, 0.54 and 0.59 among Asian, African and Caucasian respectively. A sample from British population revealed a frequency of 0.60 and 0.40 for G and C allele respectively (Timpson et al, 2007) and 0.36 and 0.64 respectively in USA sample (Kim et al, 2003).

TAS2R38 (p49A) genotypes in present Basra population sample showed a frequency of 0.17, 079 and 0.07 for CC, CG and GG respectively. These result was in contrary to a mixed sample from Indian, Chinese and Malaysian where GG recorded a frequencies of 0.35, 0.38 and 0.05 respectively (Ooi *et al*, 2010). Whereas, PAV/AVI (CG), PAV/PAV (CC) and AVI/AVI (GG) genotypes in Turkish population samples showed frequencies of 0.236, 0.466 and 0.298 respectively (Ergun and Askoy, 2013). Those of Hawaii USA were 0.256, 0.466 and 0.278 respectively (Schembr *et al*, 2013). Different world population differ in genotypes frequencies of this gene due to different race and religion (Ooi *et al*, 2010 and Schembre *et al*, 2013) as well as geography (Olivera, 2012).

In conclusion, it is worth mentioned here that TAS2R38 gene has other rare alleles did not appeared here. This result is similar to that of Behrens et al (2013). On the other hand, Campbell *et al* (2012) reported a 21 SNPs to this gene among Africans, 19 (95%) of these SNPs showed replacing of amino acids which indicates a high and effective natural selection pressure on this trait among human (Campbell *et al*, 2012). All these SNPs have been discovered in Africa, whereas, Caucasian and Asian were less diverse as PAV and AVI alleles contributed by >90% in the total variation of PTC taste, while among African contributed by 80% only (Campbell *et al*, 2012).

References

Bazzan AJ, Newberg AB, Cho WC and Monti DA. (2013) Diet and nutrition in cancer survivorship and palliative care. Evidence-Based Complementary and Alternative Medicine, 2013:1-12.

Bufe B, Breslin PA, Kuhn C, Reed DR, Tharp CD , Slack JP, Kim UK, Drayna D. and Meyerhof W. (2005).The molecular basis of individual differences in phenylthiocarbamide and propylthiouracil bitterness perception. Curr Biol, 15:322-327.

Behrens M, Gunn HC, Ramos PC, Meyerhof W and Wooding SP. (2013). Genetic, Functional, and Phenotypic Diversity in TAS2R38- Mediated Bitter Taste Perception. Chem. Senses, 38: 475–484,

Campbell MC, Ranciaro A, Froment A, Hirbo J, Omar S, Bodo J, Nyambo T, Lema G, Zinshteyn D, Drayna D, Breslin PAS. and Tishkoff SA. (2012) . Evolution of Functionally Diverse Alleles Associated with PTC Bitter Taste Sensitivity in Africa. Mol Bio Evol, 29 (4): 1141-1153.

Duffy VB , Davidson AC, Kidd JR, Kidd KK, Speed WC, Pakstis AJ, Reed DR, Snyder DJ, Bartoshuk LM. (2004). "Bitter receptor gene (TAS2R38), 6-n-propylthiouracil (PROP) bitterness and alcohol intake.

Alcohol Clin Exp Res, 28(11): 1629-1637.

Ergun C. and Askoy M. (2013). Relationships between the *htas2r38* genotype, food choice, and anthropometric variables in normal-weighted and overweight adults. Genetika, 45 (2): 381-391.

Fogg-Johnson N. and Kaput J. (2003).Nutrigenomics: An emerging scientific discipline. Food Technology, 57(4): 60-67.

Fox AL. (1932). The relationship between chemical constitution and taste. Proceedings of the National Academy of Sciences, 18:115–120.

Kim UK, Jorgenson E , Coon H , Leppert M , Risch N. and Drayna D. (2003). Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. Science, 299:1221-1225.

Kaput J. and Morine M. (2012). Discovery-Based Nutritional Systems Biology: Developing of Nutrigenomic Research. Int. J. Vitam. Nutr. Res., 82 (5): 333 – 341.

Meyerhof W , Batram C , Kuhn C , Brockhoff A , Chudoba E , Bufe B, Appendino G . and Behrens M. (2010).The molecular receptive ranges of human TAS2R bitter taste receptors. Chem Senses. 35:157–170.

Oliveira MSS. (2012). A genetic approach to the relationship between taste perception and lifestyle in Africa. MSc. Thesis University of Porto, Portugal.

Ooi SX, Lee PL, Law, HY. and Say YH. (2010). Bitter receptor gene (*TAS2R38*) P49A genotypes and their associations with aversion to vegetables and sweet/fat foods in Malaysian subjects. Asia Pac J Clin. Nutr, 19 (4):491-498.

Padmavathi M. (2013). A Study on Phenylthiocarbamide Tasting in Bagatha Tribes in India". Int. Res. J. Biological Sci, 2(4), 33-36.

Schembre SM, Cheng I, Wilkens LR, Albright CL. and Marchand LL. (2013). Variations in Bitter-Taste Receptor Genes, Dietary Intake, and Colorectal Adenoma Risk. Nutri. & Cancer 65(7): 982-990.

Timpson NJ, Heron J, Day IN. (2007). Refining associations between TAS2R38 diplotypes and the 6- n-propylthiouracil (PROP) taste test: findings from the Avon Longitudinal Study of Parents and Children. BMC Genet, 8:51.

Turner-McGrievy GF, Tate D. and Moore D. (2013). Taking the bitter with the sweet: Relationship of super tasting and sweet preference with metabolic syndrome and dietary intake. J Food Sci., 78(2): S336–S342.

Wooding S, Kim UK, Bamshad MJ, Larsen J, Jord L. and Drayan D. (2004). Natural selection and molecular evolution in PTC bitter-taster receptor gene. Am. J. Hum. Genet. 74: 637– 646.

Wooding S, Atanasova PS, Gunn HC, Staneva R, Dimova I. and Toncheva D. (2012). Association of a bitter taste receptor mutation with Balkan Endemic Nephropathy (BEN). BMC Medical Genetics, 13:96-111.