Polymorphisms within the ptc gene

The capability to taste phenylthiocarbamide (PTC), which is a bitter chemical that has been recognized as a heritable trait and has been also widely used for b genetics and anthropological studies. The frequency of nontaster and taster allele is found to be varied within different populations. This trait also has been revealed to associate with numbers of dietary preferences and accordingly could have significant implications for the human health. Aim and objectives: to investigate the Allele frequency of the ability to bitter taste perception of phenylthiocarbamide (PTC) by using restriction digestion (Fnu4H1) within our class group. Material and Method: the present investigation conducted by 57 paricipants from the students. This practical work then has been extended through bioinformatics to understand the process through analysing of the genetic differences. Results: it was found that tasters were more frequent with heterozygous taster allele frequency (Tt) 56. 41% and homozygous taster 8. 77% than the nontaster allele (tt)frequency 35%. Conclusion: taster are more frequent than nontaster. PTC taster is genetically inherited. This gives the food preference between people.

The human sense of taste consists of five basic tastes: sweet, sour, bitter, salty and umami. Human taste perception is verified by the number, type and structure of taste cells that found on tongue taste buds. Each taste cell has specific proteins on its surface that act as taste receptors. The ability to taste is essential for nutrition and survival for example bitter perception protect human from ingesting naturally toxic substances. Thus many studies focused on how most chemical structures and chemically classes are bitter taste (ref).

Taste perception & genetic link

Taste sensitivity varies between individuals. The variations in taste perceptions are the physiological expression (phenotype) of individuals genotypes. Scientists now knows that gene plays a critical role in the taste sense. Differences in taste-receptor genes can consequence in different amino acid sequences that provide taste-receptor proteins different shapes. Bitter perception occurs through bitter taste receptors which are encoded by T2R genes that show 25-89% amino acid sequence identity among the 25 different members of this gene family. These wide differences allow a huge variety of different chemical shapes, size and functionalities to be recognized by this receptor and taste as bitter (ref). The most known gene that has an impact on the bitter taste perception is TAS2R38 gene on chromosome 7.

PTC gene & polymorphisms

Differences in TAS2R38 gene affect whether people can taste a chemical called phenylthiocarbamide (PTC) as it encodes a taste receptor that found on the tongue. Variation in the ability to taste PTC was first recognized in the early 1930s, when Arthur Fox discovered the polymorphism in himself and a coworker, organic chemist. Subsequent studies by Albert Blakeslee, showed that the inability to taste PTC is a recessive trait that varies in the human population. The gene for the PTC taste receptor, TAS2R38, was identified in 2003 by Kim and co-workers. The inability of some individuals to taste PTC was found to separate in a nearly Mendelian recessive manner, with two different alleles, (T) for taster individual and (t) for non taster. These genetic make-up differences can be explained as genotypes. The taste ability of PTC or not to taste is known as a phenotype. Additionally, these genetic

variations can be described as single nucleotide polymorphisms (SNPs), that is only one nucleotide or letter changed within the DNA sequence of the TAS2R38 gene.

The single exon of the PTC gene encodes a G-protein linked receptor, 333 amino acids in length, with seven-transmembrane domains. Kim and co-workers identified three common SNPs associated with PTC sensitivity, each of which results in changes to the amino acid sequence and can be inherited together in certain combinations, e. g., haplotypes correlates most strongly with tasting ability. The known SNP haplotypes of the PTC gene (named for the first letter of the amino acid present at positions 49, 262 and 296) are common over 84% of all haplotypes, 41% AVI (Ala-Val-Ile) non-taster and 43% PAV (Pro-Ala-Val) taster. PAV, AVI and3. 3% AAV (Ala-Ale-Val) see Table 1.

A later screen recognized two additional haplotypes, PAI (Pro-Ale-Ile) and PVI (Pro-Val-Ile) both are extremely rare <1 % and AAI (Ala-Ale-Ile) 12. 2%, which were found only in individuals of sub-Saharan African ancestry. The AVI nontaster haplotype was found in all populations except Southwest Native Americans (Kim et al., 2003). 8

Aim of study

This study aimed to allow the students to investigate their own ability to bitter taste preceprtion of phenylothioarbamide (PTC) by using restriction digestion (Fnu4H1) and separation by gel electrophoresis. This practical work then has been extended through bioinformatics to understand the process through analysing of the genetic differences.

Methods

DNA extraction

This practical has been conducted by using buccal cheek cells samples. Cheek cells were obtained by using a sterile wooden splint and then were removed by plastic loop. Next the loop was twirled into 1. 5ml eppendrof tube which is containing 350ul 5% chelex suspension to denature the proteins and allow the DNA to be release. After that 4 ul of Proteinase K was added to the 1. 5ml eppendrof tube which contain the suspension and kept for incubation (at 56Co for 30 minutes), followed vortex for 10 sec. The tubes were centrifuged for 20 seconds at maximum speed (13, 000) rpm then were incubated at 98oC in heating block for 15 minutes to denature the protein and releasing the DNA. Again the tube was vortex for 10 seconds and then centrifuged at maximum speed for 3 minutes. Finally, the supernatant which contained buccal cells DNA transferred to 1. 5 ml sterile eppendrof tube and kept on ice to preserve the DNA.

PTC Alu PCR Reaction

44 ul of the Master mix (contains TAQ polymerase, dNTPs, buffer ddH2O, MgCl2, Forward Primer, Reverse Primer) was added to 6ul of the DNA template. The forward primer was 5’AACTGGCAGATTAAAGATCTCAATTTAT3′ and the reverse primer was 5’AACACAAACCATCACCCCTATTTT3′. Then the all components were mixed. Finally the tubes were placed in the thermal cycler and run using the following programme to allow the PCR process. First step is denature, the temperature was increased to 94C for 4 minutes to separate the DNA double stranded to a single DNA stranded. Second step is annealing at 59oC. This low temperature allows the primer to form hydrogen bonds or

annealing with their complementary sequence in the target DNA. Final step is the extension at 72oC, which is the optimal temperature for Taq polymerase. In this step new DNA strand synthesizes by DNA polymerase.. The three previous steps were repeated for 33 times which took approximately 2 hours.

94oC denature 4 minutes

Repeated for 33 cycles59oC 40 seconds

72oC annealing 40 seconds

94oC 40 seconds

59oC 5 minutes

72oC extension 5 minutes

Restriction Fragment Length Polymorphic analysis (RFLP)of PTC genotyping

From the unique PCR testing assay tube, aliquot of 10ul of PCR component mixture were transferred into new fresh tube, then 5ul of restriction Endonuclease Master mix(Fnu4H1, previously prepared) was added to the same tube. Finally, the new tube mixture was placed into the heating block at 37oC for approximately 4 hours. The original PCR tube returned back to the ice to be use later.

Electrophoresis of PCR products

After the 4 hours RFLP analysis was completed. The electrophoresis cassette was ready to use with 2% solidify agarose gel and TBE buffer was added until

it cover the top of the gel. After that the two samples prepared by adding 3 µl of loading buffer (which allow the sample to sink down when it load in the gel) and 15 µl of the sample (the first tube sample contained only the PCR product(U) and the second sample contained PCR product with restriction enzyme (D)). Then, 10ul of the PCR product/RFLP of student sample was loaded into the gel wells starting by digested sample and the next well with the undigested sample along with 10ul of the 100bp ladder loaded in the first well (see figure 1). The electrophoresis was carried on 5Vcm-1 (approx 70V) for 1 hour. After that, the gel was stained with ethidium bromide for 10 minutes and it destined for another 10 minutes in water. Finally, the gel were kept under UV transillumination and the photograph were printed.

Results:

Figure 2: This gel was from my own group including me and two other students. IT was run to verify PTC genotype. Three different sets of samples were used student 1 (lane 2 and 3), student 2 (lane 4 and 5), student 3 (lane 6 and 7). Lane 1: contains 100 bp ladder, Lane2 (undigested) and 3 (digested): has only one band in position 302bp (non-taster), Lane 4 (undigested): showed only 1 band, Lane 5 (digested): shows three bands in positions 302bp and 251bp but the third band was faint but it is in position 51bp (heterozygous taster) , Lane 6 (undigested) and 7 (digested) : has only one band in position 302bp (non-tester).

To determine the size of the fragment, the equation from the standard curve applied as following:

The equation is Y= [-0. 4057(x)] + 3. 7842, (x) is the distance in cm(distance measurement started from the well to the band)

From the gel all undigested band which is the (tt) homozygous recessive distance is 3. 2 cm so all bands should give the same molecular weight by applying the equation

Y= [-0. 4057 x In(3. 2)] + 3. 7842 = 2. 48

Therefore, anti-log(2. 48) = 302bp

The three fragments distance obtained from the digested (Tt) sample are 3. 2cm , 3. 4cm and the third fragment was faint and difficult to obtain but it most probably at 5. 1cm. By applying the equation, the first fragment distance of 3. 2 will give the same result of 302bp. The second fragment distance is 3. 4cm, so:

Y= [-0. 4057 x In(3. 4)] + 3. 7842 = 2. 4

Therefore, anti-log(2. 4) = 251bp

The third fragment distance is 5. 1cm:

Y= [-0. 4057 x In(5. 1)] + 3. 7842 = 1. 71

Therefore, anti-log(1. 71) = 51bp

By using the primer sequences & BLAST programme, the cDNA (RNA) sequence of the PTC gene was obtained from pubmed (http://blast. ncbi. nlm. nih. gov/Blast. cgi):

DEFINITION: Homo sapiens taste receptor, type 2, member 38 (TAS2R38), mRNA.

ACCESSION: NM_176817

SOURCE: Homo sapiens (human)

This gene is located in chromosome: 7; Location: 7q34. The position of the forward primer and the reverse PCR primer are shown in green and yellow, respectively.

The forward primer is 5’AACTGGCAGATTAAAGATCTCAATTTAT3′ starts from 631bp to 658bp.

The reverse primer is 5’AACACAAACCATCACCCCTATTTT3′ starts from 910bp to 933bp.

The complementary strand for the reverse primer is

5′ AAAATAGGGGTGATGGTTTGTGTT3′

Restriction site for Fnu4H1:

The Fnu4H1 site that used for genotyping is underlined and colored in blue at position 869bp.

Restriction site for Fnu4H1:

The recognition site for the enzyme Fnu4H1 is 5′—GCTGC—3′ which cleaves the tasters (TT/Tt) sequence as following:

In case of homozygous taster(TT) there will be 2 fragments will be detected which is not shown in our gel as none of us the three students homozygous dominant taster

Fragment one: consist of 251bp (starting from the beginning of the forward primer (631) to the Fnu4H1 restriction site).

Fragment two: consist of 51bp (starting after the Fnu4H1 restriction site to the end of reverse primer (933)). The homozygous tasters PCR product generated with the restriction enzyme is as show in figure2.

In heterozygous tasters (Tt), three fragments were produced because it combines the both alleles, one from the homozygous tasters (T) and the other from the homozygous non-tasters (t). Therefore, as it was shown in our gel the end product will have three fragments, two from the homozygous tasters, 251bp and 51bp, and one from the homozygous non-tasters, 302bp, which means the second student (my sample) is (Tt) heterozygous taster.

When Fnu4H1 was added to non- tasteer sequence, one fragments was obtained as no cleavage occured:

In homozygous non-tasters (tt), the Alanine (A) amino acid is replaced by Valine (V) because the Cytosine (C) is substituted to Thymine (T), which is known as Single Nucleotide Polymorphism (SNP). Thus, duo to this SNP, the sequence will change from 5′—GCTGC—3′ to 5′—GTTGC—3′ and the Fnu4H1enzyme will not cut the sequence. The PCR product produced by Fnu4H1 is explained in figure no (3).

In picture of our gel, a single band has been detected in the position of 302bp in both undigested and digested lanes of sample one and sample 3, which means the first student and the third student are (tt) homozygous non-taster because of the absence of the allele as no digestion occur by restriction enzyme. Thus only one fragment was produced with 302bp.

The SNP at position 869 is useful for determining the PTC genotype as the taster sequence in this region makes a Fnu4H1 restriction site by replacing C869 with T869 in the non-taster allele eliminaste this restriction site. A restriction site known to be a specific DNA sequence that recognized by a particular restriction enzyme (endonuclease)and cleaved. We have used primers particularly designed to attach to regions nearby the one polymorphic Fnu4H. By using these primers in a polymerase chain reaction (PCR) generates a 302bp fragment. Restriction digestion of the PCR product with the Fnu4H1 will yield one 302 bp fragment for the non-taster allele and two shorter fragments (51 bp and 251 bp) for the taster allele.

Discussion

Our class allele frequency study of 57 students from different populations (Europe, Asia & Africa) revealed that 5 of students (8. 7%) were Homozygous taster (TT), 32 of students (56. 3%) Heterozygous tester (Tt) and 20 students (35%) were Non-tester (tt). The class results for PTC tasting ability were quite variable. Most people’s data indicated that they are partial tasters (Tt heterozygous) or full taster (homozygous taster TT) , but there were considerable numbers of people who were non-taster (heterozygous tt) .

These obtained results reflect a slightly lower percentage of our class allele (TT) frequency than that reported from other studies done among European population and other population. NCBI reported on previous conducted study among European (Western and Northern) population done for the International al HapMap project included 226 participant, their allele frequency were as followed homozygous tester (TT) 33. 6%, Heterozygous (Tt) 46. 9% and Non-tester (tt) 19. 5%. Moreover, approximately similar prevalence of (Tt) allele has been noted in our class participant compared to European population but the percentage of non-taster(tt) allele is more than the percentage found with European population. The difference in prevalence of taster to non taster ratio is higher than reported among our class allele frequency .

On the other hand, when comparing allele frequency of our class participant results with a study that has been done previously on sub Sahara African which is also done for the International al HapMap project included 224 samples, 8% of sub Sahara African were taster (TT), 47. 3% were homozygous taster (Tt) and 44. 6% of them were not taster. The percentage of taster (TT) allele is approximately similar to our class (TT) allele frequency but the (Tt) percentage in our class frequency allele is more than what was determined in this study. In addition, the difference in prevalence of taster to non taster ratio is approximately similar to our class allele frequency.

Moreover, Scientists have found that the bitter taste perception is associated to human populations that share common genes. For example, the percentage of people who do not taste bitterness ranges from 3% in West

Africa; 6 to 23% in China; 40% in India and approximately 30% in European decent people.

Additionally, TAS2R38 haplotypes have been reported to influence food preferences, as this gene is controlling the taste ability of the glucosinolates, a family of bitter tasting compounds that are extensively distributed in the Brassica sp plants. PTC and 6-n-propylthiouracil (PROP) both have been recognized as major ligands for this receptor (3). A rising literature proposes that the ability of tasting PTC/PROP can influence dietary behavior. The differences in PTC/PROP taste sensitivity has been linked with variation in preferences and vegetables and bitter fruits selection, as well as spicy foods, sweet foods, alcoholic drinks and added fats, and. The perceived bitterness of PROP has also been associated with drinking behaviours and oral sensation. A positive association was observed between Maxdrinks and TAS2R38 haplotypes in Collaborative Study on the Genetics of Alcoholism (COGA) high risk women of African-American derivation. A lot studies show that though nontasters are less expert at discerning fat, they favor higher fat meats, cheese and milk more than the tasters. Moreover, high bitter concentration compound lead to food preference or food rejection. This can draw to avoid eating of many toxics contained in food, for example hydrolyzed protein and rancid fat.

Genetically and geographically isolated populations provide different advantages for studying the genes influence on multifaceted diseases such as obesity. Fisher et al have found that PTC taster people are thinner than the non taster [5], since they show more sensitivity to fat texture [4], while non- tasters could not differentiate between high/low fat contents as they

have less taste buds on their tongue [5]. Moreover, there is a study suggests that people who can taste bitter flavors are less likely to smoke as nicotine has bitter taste. Smokers are less likely to taste PTC than nonsmokers. The stusy found that 80% of the nonsmokers can taste PTC while 64% of the smokers could taste PTC.

Totally there are 25 human bitter receptors, and their particular genes (TAS2Rs) contain high allelic variation levels, which might influence the response to bitter compounds in the food . The ability to taste PTC is a typical phenotype, which is of genetic, evolutionary and epidemiologic interest as PTC taste ability is linked with the ability to taste other bitter substances, a lot of which are toxic. Thus, different PTC perception may reflect variation in dietary preferences among human, that may have major health effects and can associate with diet-related diseases susceptibility in modern populations. Although in nature the PTC itself has not been found, it is related structurally to a group of compounds that found in cruciferous vegetables (e. g broccoli, cabbage and Brussels sprouts) which are toxic in large quantities, with the thyroid as the primary organ affected. For examples, iodine deficiency can cause thyroid disease as it is a molecule in the thyroid hormone. Hence, natural selection of food is related to the amount of iodine in the diet [13]. Furthermore, in low iodine geographical regions, isothiocyanates over-ingestion has been linked with thyroid disease. Isothyocyanates and goitrin are bitter PTC-related compounds caused by hydrolysis of glucosinolates naturally present in raw cabbage and thiocarbamide derivatives which work as thyroid inhibitors. Usually these compounds are rejected by PTC tasters, thus the non tasters people are

more likely to have thyroid deficiency [11]. Therefore, differences in the TAS2R38 gene may be beneficial in preventing or allowing the ingestion of isothiocyanates without experiencing an unpleasant taste. In addition, brassica vegetables such as broccoli and sprouts brussel include isothiocyanates which work as a ligand for TAS2R38 receptor. These vegetables have been shown to have strong anti-cancer effects, and are therefore beneficial to eat but people who have sensitivity to bitter taste may avoid these healthy vegetables, which is rich in anti tumor and anti oxidant compounds. Thus, because of their rejection to bitterness and their encouragement to sweets and fatty foods(like cruciferous vegetables and fat foods); these people are more likely to be at risk from obesity, cardiovascular diseases risk factors and cancer.

There is another disease that many studies suggest that may has a relation with PTC gene. Some data suggest that PTC non taster frequency increase in Parkinson Disease (PD). These suggest that non tasters are at risk to develop Parkinson disease [14].

Finally, PTC provides a unique prospect in bitter taste transduction field. Having a known gene with a strong effect on phenotype in vivo provides many opportunities for studies of taste physiology, biochemical function, and molecular structure elucidation in the human sense of taste. Another use of SNPs is the ability to compare the possible SNPs between populations of those who have a disease and those who do not of the disease.

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