Bitter taste perception in Neanderthals through the analysis of the \textit{TAS2R38} gene

Carles Lalueza-Fox$^{1,4}$, Elena Gigli$^1$, Marco de la Rasilla$^2$, Javier Fortea$^2$ and Antonio Rosas$^3$

1. INTRODUCTION

Sensory response to bitter taste has an important role in the regulation of the intake of some toxic substances in food, that otherwise could produce poisoning. Therefore, this trait is likely to have been important in the dietary and ecological strategies of past human groups. Bitter taste is mediated by G-protein-coupled receptors expressed in taste cells on the surface of the tongue. These proteins are encoded by the \textit{TAS2R} gene family. Inter-individual variability related to bitterness perception is associated with genetic variation in these genes (Drayna 2005).

The most extensively studied taste variation in humans is sensitivity to a bitter substance called phenylthiocarbamamide (PTC). Although approximately 75 per cent of the world population perceives this substance as intensely bitter, it is virtually tasteless for the remaining 25 per cent of the population (Kim & Drayna 2004). This is owing to a dominant ‘taster’ allele that shows a similar frequency to the recessive ‘non-taster’ allele. PTC itself is not found in any vegetable, but chemically similar substances that produce an identical response to PTC are present in many plant foods (including Brussels sprouts, cabbage, broccoli and others). It was discovered (Kim et al. 2003) that most of the variation in PTC sensitivity is related to polymorphisms at the \textit{TAS2R38} gene, a single 1002 bp coding exon that encodes a 333-amino-acid, G-protein-coupled receptor. The \textit{TAS2R38} gene has three amino-acid changes in high frequencies that determine only five main haplotypes. These polymorphisms are found at position 49 (encoding proline or alanine), 262 (alanine or valine) and 296 (valine or isoleucine) of the gene. The two most common haplotypes are proline–alanine–valine (PAV) and alanine–valine–isoleucine (AVI). PAV is the major taster haplotype (carrying one or two copies), and AVI is the major non-taster haplotype (carrying two copies). AVI/PAV heterozygotes have slightly less sensitivity to PTC (Drayna 2005). Of the three common variants, the nucleotide substitution at the amino-acid residue 49 (A49P) shows the strongest association with taster status; a proline in that position always implies PTC taste perception (Drayna 2005).

Perception of taste is important for nutrition and health, both in current and ancient hominin species (Drayna 2005). Neanderthals (\textit{Homo neanderthalensis}) were a hominine species living in Europe and Western Asia from approximately half a million years ago to approximately 28 000 years ago (Stringer & Gamble 1999). It is therefore of interest to determine if the variation observed in the taste perception of modern humans also existed among Neanderthals.

2. MATERIAL AND METHODS

El Sidrón is a karstic system in Asturias (north of Spain) where the remains of 11 Neanderthal individuals (dated to about 48 000 years ago; Santamarina et al. in press) have been excavated since 2000 (Rosas et al. 2006). The remains are retrieved under controlled conditions (e.g. excavators wear sterile laboratory gear, face masks, sterile gloves and so on) and are immediately frozen before being shipped to the paleogenetic laboratories. This procedure has allowed the reduction of exogenous DNA contamination from very high ratios to almost negligible figures (Fortea et al. 2008).

An adult male bone flake, labelled as SD 1253, is one of the best El Sidrón samples in terms of genetic content and low DNA contamination. In a recent massively parallel sequencing approach for this sample, only 0.27 per cent of the mitochondrial sequences obtained were exogenous contaminants (Briggs et al. 2009), the lowest level for any Neanderthal sample studied so far. In addition, some nuclear genes related to language capacity (Krause et al. 2007), pigmentation (Lalueza-Fox et al. 2007) and blood group type (Lalueza-Fox et al. 2008) have been retrieved from this and other El Sidrón samples in recent years.
Figure 1. 454 pyrosequencing results for the El Sidrón 1253 TAS2R38 gene at the 49 amino-acid position. C, taster (proline amino acid); G, non-taster (alanine amino acid). Number of total sequences in brackets.

3. RESULTS
A total of 4307 sequences were generated for the TAS2R38 gene (figure 1). Of the total, 2391 (55.51%) showed a C in nucleotide position 145, corresponding to a proline amino acid (taster haplotype), and 1916 showed a G (44.49%), corresponding to a proline amino acid (taster haplotype). Several primer pairs were designed to amplify the three polymorphic amino-acid positions that determine the common TAS2R38 haplotypes, although only one couple (F142, 5′-TTGGATGTAGTGAAGAGGCAGSCACTGAGCAACAGTGATTGTGTGCTGCTGTGTCTCAGCAT-3′; R166, 5′-ATGCTGAGA-CAGACGCCGAC-3′) yielded a band of the correct size (63 bp). The PCR product was visualized in a low-melting-point agarose gel, excised from it and purified using a silica binding method. Subsequently, it was massively parallel sequenced at the Centre de Regulación Genómica (CRG) of Barcelona using the 454 GS-FLX platform along with other PCR products (C. Lalueza-Fox 2009, unpublished data). TAS2R38 gene sequences were subsequently identified and aligned. To monitor for potential exogenous DNA contamination, one Y-chromosome marker (Y2 in Krause et al. 2007) was co-amplified along with the TAS2R38 polymorphism. It was previously known that El Sidrón 1253 showed the ancestral allele in the Y-chromosome. This position defines the deepest clade in the human Y-chromosome tree and it is currently found only in some sub-Saharan African groups (Krause et al. 2007).

3. RESULTS
A total of 4307 sequences were generated for the TAS2R38 gene (figure 1). Of the total, 2391 (55.51%) showed a C in nucleotide position 145, corresponding to a proline amino acid (taster haplotype), and 1916 showed a G (44.49%), corresponding to an alanine amino acid (non-taster haplotype). Three clones show singleton C to T or G to A substitutions that are the most common form of post-mortem DNA damage (Briggs et al. 2007). The main researcher involved in the laboratory analysis (C.L.-F.) is proline homozygous. All the Y-chromosome sequences identified (n = 141) showed the ancestral allele and, thus, no male contamination of European origin could be detected in this amplification.

Because almost half of the TAS2R38 clones showed either one or another nucleotide, it is most likely that the El Sidrón 1253 individual was A49P heterozygous. Therefore, this Neanderthal was a taster individual, although slightly less of a taster than an amino-acid-49 proline homozygote (Drayna 2005). We have not been able to obtain any amplification for the two other amino-acid positions and, therefore, do not know the complete Neanderthal haplotype for the TAS2R38 gene.

4. DISCUSSION
The existence of taster and non-taster individuals in chimpanzees has been known since 1939 (Fisher et al. 1939). It has been presumed that humans and chimpanzees shared the balanced polymorphism associated with PTC tasting ability and that this evolved before the divergence of both lineages (Wooding et al. 2004). However, it has recently been discovered that the trait is controlled in chimpanzees by two common alleles at the TAS2R38 locus that are not shared with humans, and thus the non-taster alleles have evolved at least twice during hominin evolution (Wooding et al. 2006).

From modern sequence data, the divergence time of the two common TAS2R38 haplotypes has been estimated to be approximately 1.5 million years, although with a large margin of error (Wooding et al. 2004). Owing to the improbability that A49P mutation could be homoplastic, the most plausible explanation for the observation of a Neanderthal A49P heterozygote is that this polymorphism pre-dates the split of Neanderthal and modern human lineages, known to have been at least half a million years ago (Noonan et al. 2006; Green et al. 2008). If we discard the unlikely scenario of gene flow between both human groups (Krause et al. 2007) around the time of the arrival of modern humans to Europe (approx. 40 000 years ago), our results indicate that the non-taster alleles were already present in the ancestral human populations from which both Neanderthals and modern humans diverged.

The high frequency of the non-taster allele in humans is surprising, because the bitter taste sense is assumed to protect against the ingestion of toxic bitter substances. It has been suggested that balancing natural selection underlies the maintenance of divergent taste alleles in modern human populations favouring heterozygotes (Kim & Drayna 2004; Wooding et al. 2004). This could explain why the non-taster haplotypes do not carry mutations involving obvious loss of function (Kim & Drayna 2004). However, Wang et al. (2004) argued that the results favouring balancing selection at the TAS2R38 locus could be influenced by the demographic expansion model assumed by Wooding et al. (2004). Our results indicate that, whatever the environmental factors and selective pressures that may be acting upon this gene to maintain this polymorphism, they were also present in the Eurasian environments where the Neanderthals lived.

This work has been financed by a grant from the Ministerio de Ciencia e Innovación from Spain.


Bitter taste perception in Neanderthals

C. Lalueza-Fox et al.


