The bitter taste perception (associated with the ability or inability to taste phenylthiocarbamide) is mediated by the \( \text{TAS2R38} \) gene. Most of the variation in this gene is explained by three common amino-acid polymorphisms at positions 49 (encoding proline or alanine), 262 (alanine or valine) and 296 (valine or isoleucine) that determine two common isoforms: proline–alanine–valine (PAV) and alanine–valine–isoleucine (AVI). PAV is the major taster haplotype (heterozygote and homozygote) and AVI is the major non-taster haplotype (homozygote). Amino acid 49 has the major effect on the distinction between tasters and non-tasters of all three variants. The sense of bitter taste protects us from ingesting toxic substances, present in some vegetables, that can affect the thyroid when ingested in large quantities. Balancing selection has been used to explain the current high non-taster frequency, by maintaining divergent \( \text{TAS2R38} \) alleles in humans. We have amplified and sequenced the \( \text{TAS2R38} \) amino acid 49 in the virtually uncontaminated Neanderthal sample of El Sidrón 1253 and have determined that it was heterozygous. Thus, this Neanderthal was a taster individual, although probably slightly less than a PAV homozygote. This indicates that variation in bitter taste perception pre-dates the divergence of the lineages leading to Neanderthals and modern humans.

**Keywords:** bitter taste; PTC; \( \text{TAS2R38} \); Neanderthals

1. INTRODUCTION

Sensitivity 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 \( \text{TAS2R} \) gene family. Inter-individual variability related to bitterness perception is associated with genetic variation in these genes (Drayna 2005).
The El Sidron 1253 sample was extracted using a previously established protocol, based on proteinase K digestion, phenol-chloroform extraction and centric microcolumn concentration (Lalueza-Fox et al. 2005, 2006). Two-step multiplex polymerase chain reactions (PCRs, Krause et al. 2006) using a total volume of 20 µl were set up, and included 1× Taq Gold buffer, 2.5 mM MgCl2, 1 mg ml⁻¹ bovine serum albumin, 300 µM of each nucleotide, 150 nM of each primer and 2 units of Taq Gold DNA polymerase. The first and second PCR reactions consisted of 27 and 33 cycles, respectively, using 55 ºC as the annealing temperature.

The concentration of the primers was increased to 1.5 µM in the second PCR reaction. 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′-TTGGGATGTAGTGAAGAGGC-3′; R166, 5′-ATGCTGAGACAGGACGCAC-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 Sidron 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 genotyped for the TAS2R38 gene F142-R166 fragment (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 postmortem DNA damage (Briggs et al. 2007). The main researcher involved in the laboratory analysis (C.L.-F.) is 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.

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