

Bitter taste perception in Neanderthals through the analysis of the *TAS2R38* gene

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The bitter taste perception (associated with the ability or inability to taste phenylthiocarbamide) is mediated by the *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 *TAS2R38* alleles in humans. We have amplified and sequenced the *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; *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 *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 phenylthiocarbamide (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 *TAS2R38* gene, a single 1002 bp coding exon that encodes a 333-amino-acid, G-protein-coupled receptor. The *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). Other additional haplotypes, AAV, PVI and AAI, which are presumably recombinants of the two common isoforms, have been found at lower frequencies (Drayna 2005). These minor haplotypes, as well as two additional rare variants at positions 80 and 274, are basically found in sub-Saharan African populations (Wooding *et al.* 2004).

Perception of taste is important for nutrition and health, both in current and ancient hominin species (Drayna 2005). Neanderthals (*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; Santamaría *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.

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TTGGGATGTAGTGAAGAGGCAGSCACTGAGCAACAGTGATTGTGTGCTGTGTCTCAGCAT
S1 (2390)      ..C.....
S2 (1)        ..C.....A.....
S3 (1914)     ..G.....
S4 (1)        ..GT.....
S5 (1)        ..G..T.....

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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.

The El Sidrón 1253 sample was extracted using a previously established protocol, based on proteinase K digestion, phenol-chloroform extraction and centricon 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 μ l were set up, and included 1 \times Taq Gold buffer, 2.5 mM MgCl₂, 1 mg ml⁻¹ bovine serum albumin, 500 μ M 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°C as the annealing temperature. The concentration of the primers was increased to 1.5 μ M 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'-ATGCTGAGACACAGCAGCAC-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 Regulació Genòmica (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 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 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 hominid 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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Briggs, A. W. *et al.* 2007 Patterns of damage in genomic DNA sequences from a Neanderthal. *Proc. Natl Acad. Sci. USA* **104**, 14 616–14 621. (doi:10.1073/pnas.0704665104)

Briggs, A. W. *et al.* 2009 Targeted retrieval and analysis of five Neanderthal mtDNA genomes. *Science* **325**, 318–321. (doi:10.1126/science.1174462)

- Drayna, D. 2005 Human taste genetics. *Annu. Rev. Genom. Hum. Genet.* **6**, 217–235. (doi:10.1146/annurev.genom.6.080604.162340)
- Fisher, R., Ford, E. & Huxley, J. 1939 Taste-testing in anthropoid apes. *Nature* **144**, 750. (doi:10.1038/144750a0)
- Fortea, J., de la Rasilla, M., García-Taberner, A., Gigli, E., Rosas, A. & Lalueza-Fox, C. 2008 Excavation protocol of bone remains for Neanderthal DNA analysis in El Sidrón cave (Asturias, Spain). *J. Hum. Evol.* **55**, 353–357. (doi:10.1016/j.jhevol.2008.03.005)
- Green, R. E. *et al.* 2008 A complete Neanderthal mitochondrial genome sequence determined by high-throughput sequencing. *Cell* **134**, 416–426. (doi:10.1016/j.cell.2008.06.021)
- Kim, U. K., Jorgenson, E., Coon, H., Leppert, M., Risch, N. & Drayna, D. 2003 Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. *Science* **299**, 1121–1225. (doi:10.1126/science.1080190)
- Kim, U. K. & Drayna, D. 2004 Genetics of individual differences in bitter taste perception: lessons from the PTC gene. *Clin. Genet.* **67**, 275–280. (doi:10.1111/j.1399-0004.2004.00361.x)
- Krause, J. *et al.* 2006 Multiplex amplification of the mammoth mitochondrial genome and the evolution of Elephantidae. *Nature* **439**, 724–727. (doi:10.1038/nature04432)
- Krause, J. *et al.* 2007 The derived FOXP2 variant of modern humans was shared with Neanderthals. *Curr. Biol.* **17**, 1908–1912. (doi:10.1016/j.cub.2007.10.008)
- Lalueza-Fox, C. *et al.* 2005 Neanderthal evolutionary genetics: mitochondrial DNA data from the Iberian Peninsula. *Mol. Biol. Evol.* **22**, 1077–1081. (doi:10.1093/molbev/msi094)
- Lalueza-Fox, C. *et al.* 2006 Mitochondrial DNA of an Iberian Neanderthal suggests a population affinity with other European Neanderthals. *Curr. Biol.* **16**, R629–R630. (doi:10.1016/j.cub.2006.07.044)
- Lalueza-Fox, C. *et al.* 2007 A melanocortin 1 receptor allele suggests varying pigmentation among Neanderthals. *Science* **318**, 1453–1455. (doi:10.1126/science.1147417)
- Lalueza-Fox, C. *et al.* 2008 Neanderthal paleogenomics in the ABO blood group gene. *BMC Evol. Biol.* **8**, 342. (doi:10.1186/1471-2148-8-342)
- Noonan, J. P. *et al.* 2006 Sequencing and analysis of Neanderthal genomic DNA. *Science* **314**, 1113–1118. (doi:10.1126/science.1131412)
- Rosas, A. *et al.* 2006 Paleobiology and comparative morphology of a late Neanderthal sample from El Sidrón, Asturias, Spain. *Proc. Natl Acad. Sci. USA* **103**, 19266–19271. (doi:10.1073/pnas.0609662104)
- Santamaría, D. *et al.* In press. The technological and typological behaviour of a Neanderthal group from El Sidrón cave (Asturias, Spain). *Archaeometry*.
- Stringer, C. & Gamble, C. 1999 *In search of the Neanderthals*. London, UK: Thames and Hudson.
- Wang, X., Thomas, S. D. & Zhang, J. 2004 Relaxation of selective constraint and loss of function in the evolution of human bitter taste receptor genes. *Hum. Mol. Genet.* **13**, 2671–2678. (doi:10.1093/hmg/ddh289)
- Wooding, S., Kim, U. K., Bamshad, M., Jorde, L. & Drayna, D. 2004 Natural selection and molecular evolution in PTC, a bitter taste receptor gene. *Am. J. Hum. Genet.* **74**, 637–646. (doi:10.1086/383092)
- Wooding, S. *et al.* 2006 Independent evolution of bitter-taste sensitivity in humans and chimpanzees. *Nature* **440**, 930–934. (doi:10.1038/nature04655)