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ORIGINAL ARTICLE

# Thr92Ala polymorphism in the type 2 deiodinase gene: an evolutionary perspective

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## Abstract

**Purpose** In the past, a role of thyroid hormones in human evolution has been hypothesized. T3, the metabolically active form, derives from extrathyroidal conversion of T4 by deionidase 2 (D2) enzyme encoded by *DIO2* gene. In thyroid-deficient patients, decreased levels of free T3 have been associated with the polymorphism rs225014 A/G in *DIO2*, which causes the substitution of Threonine with Alanine (p.Thr92Ala) at protein level.

**Methods** We compared DNA and protein sequences of D2 from archaic human subspecies with those of contemporary humans.

**Results** Neanderthals and Denisovans displayed only the G allele at the rs225014 polymorphism, which encodes for an Alanine on the amino acid level. These data suggest that these hominines were homozygous for the Ala amino acid. These arcaic humans often lived in condition of iodine deficiency and thus, defective mechanisms of T3 biosynthesis could be life threatining. A reduced D2 activity is likely to cause decreased T3 levels, which could be critical for those individuals. Neanderthals and Denisovans were hunters/gatherers, and their diet was mainly based on the consumption of meat, with a low intake of carbohydrates. The need for circulating T3 is reduced at such alimentary conditions. On the basis of our genome comparisons the A allele, corresponding to Threonine and associated with higher levels of circulating T3 in thyroid-deficient patients, appeared for the first time during evolution in Anatomically Modern Humans during the Upper Pleistocene and has been conserved during the Neolithic age. With the advent of agriculture and herding, individuals carrying A allele might have a higher probability for surviving and reproducing. Thus, the variant was positively selected during the evolution.

**Conclusion** Here we present an evolutionary perspective for p.Thr92Ala variant of D2 from Neanderthals to Anatomically Modern Humans

**Keywords** Deionidase type 2 · rs225014 · T3 level · Neanderthals · Denisovans · Upper paleolithic

## Introduction

Hormones are key regulators of human physiology and allow the body to adapt to changing external conditions. Among hormones, thyroid hormones (THs) play a pilot role. THs [namely thyroxine (T4) and triiodothyronine (T3)] control metabolic processes needed for normal growth and development [1]. THs are involved in metabolic regulations. They correlate with body weight, stimulate both lipogenesis and lipolysis, regulate energy storage and expenditure and control thermogenesis via their activities on brain, white and brown adipose tissue, skeletal muscle, liver, and pancreas [1]. The major secreted product of the thyroid gland is T4. In contrast, T3 is secreted only in small amounts. In healthy humans, about 70% of circulating T3 is derived

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from extrathyroidal conversion of T4 [1]. Enzymes, known as deiodinases [Deiodinase 1 (D1) and Deiodinase 2 (D2)], catalyse this conversion and are responsible for the intracellular quantities of THs [2]. Deiodinases are membrane-anchored [3] seleno-protein carrying selenocysteine at the active site and this amino acid is encoded for by the UGA codon [4], which usually signals termination of translation. D2 has a higher catalytic efficiency than D1, and therefore D2 accounts for the majority of circulating T3 at normal FT4 concentration [5, 6]. D2 is encoded for by the *DIO2* gene, which is located in humans on the long arm of chromosome 14 (14q24.2–q24.3) and spans 7.5 kb.

The polymorphism rs225014 A/G, which causes a substitution of Threonine with Alanine at position 92 in the protein (p.Thr92Ala), has been associated with decreased levels of free T3 in the serum of thyroid-deficient patients [7–11]. Molecular biology studies have shown that D2-Thr and D2-Ala share a similar subcellular localization [10]. However, tranfection of D2-Ala mutant into myoblasts from Dio2-*null* mice causes a reduction of apoptosis when compared with the transfection of D2-Thr variant into the same cells. Since in proliferating myoblasts, apoptosis depends on intracellular T3 levels, these data suggest a reduced intracellular T4-to-T3 conversion by the D2-Ala [10]. In addition, it has been demonstrated that the D2-Ala adopts an altered molecular structure, leading to an accumulation of the protein in trans-Golgi and to an impaired catalytic activity [12].

THs play a major role in modulating evolutionary strategies in response to external conditions (13). In this context, the discussion on the importance of TH levels and rhythm in ancestral humans have remained an open question for many years. Human evolution has been shaped by a tight gene-environment interaction and, with the recent sequence data of ancestral human genomes [14–19], many aspects of human evolution can find novel explanations. DNA samples are now available for Anatomically Modern Humans who lived in different ages, for Neanderthals and the Asian people known as Denisovans.

Neanderthals and Denisovans are the closest evolutionary relatives of present-day humans. They share a common ancestors which then split into two regional populations [20]. Neanderthals had a long evolutionary history. Neanderthal-like characteristics were already observed in European human fossils dated back 430,000 years ago [21]. However, most of the Neanderthal fossils were dated to a time period starting 130.000 ago [22, 23] until their demise about 40,000 years ago [24]. On the other hand, Denisovans were only recently discovered. Scientists first identified Denisovan fossils in a cave in southern Siberia in 2008 [25] and more recently on the Tibetan Plateau [26]. These data suggested that this human subspecies might have reached a wide-spread distribution in Asia. Recently [27], analysis of the genome isolated from a bone of a female, who died around 90,000 years ago, revealed that she was half Neanderthal and half Denisovan. This data suggests interbreeding between the two human subspecies but the extent of such interbreeding remains unclear.

Because of the postulated developmental relevance of the thyroid hormons, the rs225014 polymorphism of the *DIO2* gene is of evolutionary interest and prompted us to compare the sequences of the *DIO2* genes of higher mammals as well as extinct and extanct human subspecies. Here we present an evolutionary perspective for p.Thr92Ala variant of D2 from Neanderthals to Anatomically Modern Humans.

## Methods

For the present study, we analysed genomic and/or the protein sequence data for the *DIO2* gene. These data were extracted from publically available genome/exome sequence databases. These data included *DIO2* sequences of *H. neanderthalensis*, *H. denisovans* and representatives of Anatomically Modern Humans (AMH) of the Upper Pleistocene and Holocen. These sequences were compared with the corresponding data of extant humans. For Neanderthals genomic DNA was isolated from bones of three individuals found in the Vindija Cave in Croatia [23]. For Denisovans a small fragment of a finger bone discovered in Southern Siberia [28, 29] was used for DNA extraction. For Upper Pleistocene Anatomically Modern Humans, we employed the sequence data of seven Iberomarusian individuals from the Grotte des Pigeons (Morocco) dated between 15.100 and 13.900 BP [30] and five individuals from the archaeological site of Sunghir (Russia) dated to about 34.000 BP [31]. Holocene archaeological data are related to 3 Later Stone Age foragers, 31 Pastoral Neolithic and 7 Pastoral Iron Age individuals from East Africa [32]. The raw sequence data were downloaded either from the Sequence Read Archive (SRA) [33] of NCBI (https://www.ncbi.nlm.nih.gov) or from the Department of Evolutionary Genetics (https://www.eva.mpg.de) and were then processed using the SRA toolkit for further analysis. A blast similarity-based search with the ncbi-blast-2.9.0+program was carried out to extract *DIO2* sequences of ancient humans [34].

Allele/genotype frequencies for contemporary humans were obtained from the Ensemble genome browser (https://www.ensembl.org). Frequencies in different extant populations were derived from the 1000 Genomes Project (https://www.internationalgenome.org), the largest public catalogue of human variation and genotype data and from The Genome Aggregation Database—gnomAD (https://gnomad.broadinstitute.org), a resource that aggregates both exome and genome sequencing data from a wide variety of large-scale sequencing projects.

## Results

In Anatomically Modern Humans, the p.Thr92Ala variant has been reported in the European population with a mean occurrence of the Ala/Ala homozygous allele of 14.1%, and of the Thr/Ala heterozygous of 40.8%. According to the Ensembl database (https://www.ensembl.org), in the rs225014 SNP the more common A allele, encoding for Thr, has a frequency of about 65.5%, whereas the less common G allele, encoding for Ala, has a frequency of about 34.5%. Geographical distributions of the rs225014 SNP alleles in different populations in the world are summarized in Fig. 1. In addition, Table 1 presents more detailed information about allele and genotype distributions in different ethnic groups.

When we aligned the DNA sequence of archaic humans with that of contemporary humans (https://www.1000genomes.org), we found that the sequenced Neanderthal and Denisovan individuals all carried the G allele at the rs225014 SNP, corresponding to the Ala amino acid at position 92 of the D2 protein (Table 2). Thus, we hypothesized that our evolutionary relatives were Ala/Ala homozygous for the D2 protein.

Moving forward in the timeline, we aligned DNA and protein sequences of Upper Pleistocene individuals with that of contemporary humans and found that in this time periode heterozygous and homozygous Thr/Thr individuals appeared for the first time in evolution (Table 2). In particular, the Moroccan sample yielded the first evidence of Thr/Thr homozygosis. When we compared the sequences of contemporary humans and Neolithic/Iron Age individuals, we found that A allele was present in herders and these data suggest homozygous Thr/Thr subjects.

To explore whether the A or the G allele represents the ancestral allele, we evaluated the *DIO2* sequences of two African great apes, bonobo (*Pan paniscus*) and chimpanzee (*Pan troglodytes*) as well as those of the orangutan (*Pongo pygmaeus*) living in South-East Asia. According to the Ensembl database, these three primates are homozygous for the G allele (Ala at protein level), which represents the ancestral allele. Table 2 presents the bonobo sequence as example. Interestingly, in mammals the G allele (encoding for Ala) is only present in primates and mustelids. On the other hand, the A allele encoding for the Thr appears to be peculiar for Anatomically Modern Humans. Different amino acids are present at this position in other mammals (Table 3).

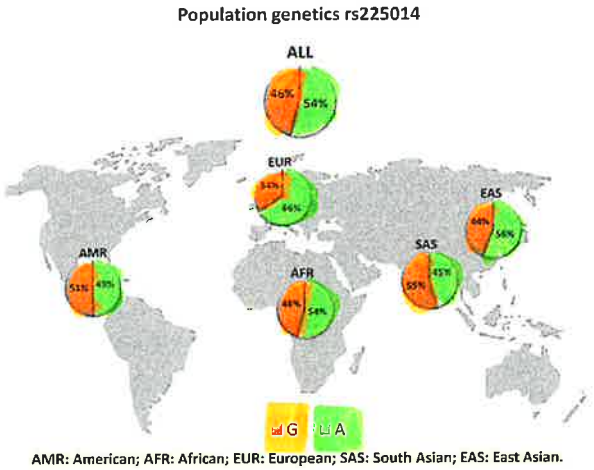



**Fig. 1** Allele frequencies for rs225014 corresponding to p.Thr92Ala. A allele encodes for Thr and G allele encodes for Ala amino acid. The overall frequency of A allele is 54% and that of G allele is 46% (Ensembl database). Considering different countries, the A allele is the most widespread globally (including Africa) except for South Asian. In The USA, frequencies are similar for A and G alleles

## Discussion

Human evolution is the result of continuous interaction of living systems with the surrounding environment and it has been modulated by the selective pressure of external stimuli on our genes [35, 36]. The change of climatic conditions, food availability, and life habits has led to the selection of genetic variants that allow a better adaptation to the environment and therefore an improved reproductive capacity. In this context, hormones as extracellular signaling molecules play important roles as mediators between genotype and phenotype. Thus, hormones must be regarded as modulators of phylogentic evolution.

The activity of the endocrine system in our ancestors, and its evolution over time, are currently subjects of great interest. Among different hormones, those produced by thyroid gland, in particular thyroxine (T4) and triiodothyronine (T3), play an essential regulatory role that links several physiological, morphological and behavioral traits, which might induce evolutionary changes. In the absence of more comprehensive information, genome analysis can provide a deeper insight into the functionality of hormones in our ancestors. Deiodinase enzymes, in particular D2, regulate the conversion of T4 to T3 and are thus responsible for the levels of circulating T3 [2, 4]. Results obtained from DNA sequencing suggested that Neanderthals possessed only the G allele in rs225014 polymorphism of the *DIO2* gene, corresponding to the amino acid Alanine at position 92 in the protein. The G allele might represent the ancestral allele, since it is present in great apes and was therefore inherited from our common ancestors. This variant is associated with a reduced catalytic activity [12] and a consequently, with a reduced capacity for T4 to T3 conversion. In patients, which

**Table 1** Geographical distributions of 225,014 SNP alleles in different populations in the world

A: 1000 Genomes Project Phase 3

| Population | Allele: frequency (count) | | Genotype: frequency (count) | | |
|---|---|---|---|---|---|
| | A | G | A \| A | G\|G | G\| A |
| All | 0.542 (2714) | 0.458 (2294) | 0.302 (756) | 0.218 (546) | 0.480 (1202) |
| AFR (African) | 0.537 (710) | 0.463 (612) | 0.277 (183) | 0.203 (134) | 0.520 (344) |
| African Caribbeans in Barbados | 0.536 (103) | 0.464 (89) | 0.271 (26) | 0.198 (19) | 0.531 (51) |
| Americans of African Ancestry in SW USA | 0.492 (60) | 0.508 (62) | 0.213 (13) | 0.230 (14) | 0.557 (34) |
| Esan in Nigeria | 0.586 (116) | 0.414 (82) | 0.354 (35) | 0.182 (18) | 0.465 (46) |
| Gambian in Western Divisions in the Gambia | 0.553 (125) | 0.447 (101) | 0.301 (34) | 0.195 (22) | 0.504 (57) |
| Luhya in Webuye, Kenya | 0.455 (90) | 0.545 (108) | 0.212 (21) | 0.303 (30) | 0.485 (48) |
| Mende in Sierra Leone | 0.547 (93) | 0.453 (77) | 0.271 (23) | 0.176 (15) | 0.553 (47) |
| Yoruba in Ibadan, Nigeria | 0.569 (123) | 0.431 (93) | 0.287 (31) | 0.148 (16) | 0.565 (61) |
| AMR (American) | 0.486 (337) | 0.514 (357) | 0.219 (76) | 0.248 (86) | 0.533 (185) |
| Colombians from Medellin, Colombia | 0.479 (90) | 0.521 (98) | 0.191 (18) | 0.234 (22) | 0.574 (54) |
| Mexican Ancestry from Los Angeles USA | 0.461 (59) | 0.539 (69) | 0.234 (15) | 0.312 (20) | 0.453 (29) |
| Peruvians from Lima, Peru | 0.406 (69) | 0.594 (101) | 0.106 (9) | 0.294 (25) | 0.600 (51) |
| Puerto Ricans from Puerto Rico | 0.572 (119) | 0.428 (89) | 0.327 (34) | 0.183 (19) | 0.490 (51) |
| EAS (East Asian) | 0.560 (564) | 0.440 (444) | 0.327 (165) | 0.208 (105) | 0.464 (234) |
| CDX; Chinese Dai in Xishuangbanna, China | 0.640 (119) | 0.360 (67) | 0.398 (37) | 0.118 (11) | 0.484 (45) |
| Han Chinese in Beijing, China | 0.524 (108) | 0.476 (98) | 0.291 (30) | 0.243 (25) | 0.466 (48) |
| Southern Han Chinese | 0.581 (122) | 0.419 (88) | 0.343 (36) | 0.181 (19) | 0.476 (50) |
| Japanese in Tokyo, Japan | 0.577 (120) | 0.423 (88) | 0.356 (37) | 0.202 (21) | 0.442 (46) |
| Kinh in Ho Chi Minh City, Vietnam | 0.480 (95) | 0.520 (103) | 0.253 (25) | 0.293 (29) | 0.455 (45) |
| EUR (European) | 0.655 (659) | 0.345 (347) | 0.451 (227) | 0.141 (71) | 0.408 (205) |
| Utah Residents (CEPH) with Northern and Western European Ancestry | 0.616 (122) | 0.384 (76) | 0.394 (39) | 0.162 (16) | 0.444 (44) |
| Finnish in Finland | 0.763 (151) | 0.237 (47) | 0.616 (61) | 0.091 (9) | 0.293 (29) |
| British in England and Scotland | 0.659 (120) | 0.341 (62) | 0.473 (43) | 0.154 (14) | 0.374 (34) |
| Iberian Population in Spain | 0.603 (129) | 0.397 (85) | 0.393 (42) | 0.187 (20) | 0.421 (45) |
| Toscani in Italy | 0.640 (137) | 0.360 (77) | 0.393 (42) | 0.112 (12) | 0.495 (53) |
| SAS (South Asian) | 0.454 (444) | 0.546 (534) | 0.215 (105) | 0.307 (150) | 0.479 (234) |
| Bengali from Bangladesh | 0.471 (81) | 0.529 (91) | 0.198 (17) | 0.256 (22) | 0.547 (47) |
| Gujarati Indian from Houston, Texas | 0.519 (107) | 0.481 (99) | 0.291 (30) | 0.252 (26) | 0.456 (47) |
| Indian Telugu from the UK | 0.392 (80) | 0.608 (124) | 0.157 (16) | 0.373 (38) | 0.471 (48) |
| Punjabi from Lahore, Pakistan | 0.443 (85) | 0.557 (107) | 0.219 (21) | 0.333 (32) | 0.448 (43) |
| Sri Lankan Tamil from the UK | 0.446 (91) | 0.554 (113) | 0.206 (21) | 0.314 (32) | 0.480 (49) |

B:

| Population | GnomAD exomes | | GnomAD exomes | |
|---|---|---|---|---|
| | Allele: frequency (count) | | Allele: frequency (count) | |
| | A | G | A | G |
| All | 0.590 (140476) | 0.410 (97626) | 0.633 (19258) | 0.367 (11176) |
| AFR (African/African American) | 0.554 (8098) | 0.446 (6514) | 0.556 (4698) | 0.444 (3752) |
| AMR (Latino) | 0.433 (14448) | 0.567 (18922) | 0.467 (385) | 0.533 (439) |
| ASJ (Ashkenazi Jewish) | 0.569 (5595) | 0.431 (4231) | 0.566 (163) | 0.434 (125) |
| EAS (East Asian) | 0.563 (9817) | 0.437 (7613) | 0.570 (874) | 0.430 (660) |
| FIN (Finnish) | 0.734 (15489) | 0.266 (5603) | 0.736 (2486) | 0.264 (894) |
| NFE (Non-Finnish European) | 0.642 (68341) | 0.358 (38165) | 0.667 (9944) | 0.333 (4968) |
| OTH (other) | 0.608 (3586) | 0.392 (2308) | 0.677 (708) | 0.323 (338) |
| SAS (South Asian) | 0.514 (15102) | 0.486 (14270) | Not available | Not available |

Population genetics for the 225,014 SNP

A: data obtained from 1000 Genomes Project Phase 3. Samples are separated into five super-populations (AFR, AMR, EAS, EUR, and SAS) and

of circulating thyroid hormones [13, 42]. Indeed, it has been shown that the levels of circulating T3, and the consequent iodine requirement, are reduced during carbohydrate deficient diet when compared with high carbohydrate diet [39, 43]. Thus, it is highly probable that the occurrence of the p.Thr92Ala variant did not make a major difference for Neanderthals and Anatomically Modern Humans since their diet was mainly based on the consumption of meat and fat. In this repsect, it is important to highlight that among the Upper Pleistocene hunter/gatherers from Morocco Thr/Thr homozygosis was observed. A shift toward a more charbohydrate-based diet has been suggested for this population: (1) the higher consumption of starch-containing plant foods [44] and (2) a higher rate of caries, which was similar to that of agricultural societies [45]. These data suggest a possible early selection pressure due to the dietary changes of these Iberomaurusian hunter-gatherers already before the advent of agriculture. It might be hypothesized that this selection pressure was intensified after Neolithization (Table 1) with the spread of agriculture and pastoralism. A diet rich in carbohydrates led to an increase in circulating T3 levels and to the need for an enhanced intake of iodine. After these changes, individuals carrying the A allele had a higher probability of surviving and reproducing and thus, this variant was positively selected. Today, the A allele is most widespread globally (including Africa), even though its frequencies are different in various parts of the world.

It is interesting to note that the rs225014 polymorphism has been associated with the risk to develop type 2 diabetes. The individual homozygous for the G allele (Ala/Ala) exhibits impaired glycemic control mechanisms and has a higher risk to develop diabetes although their serum free T3 were normal [9, 46]. It was hypothesized that a high carbohydrate diet may have positively selected the A allele to increase tissue T3 content and reduce the consequences of diabetes.

The rs225014 polymorphism might represent an example of natural selection (Fig. 2). A specific mutation that occurred by chance induced phenotypical alterations, which initially represented a "neutral" event, conferring neither an evolutionary advantage nor a disadvantage. However, with the change of external conditions (in this case the diet), individuals carrying this mutation exhibited an increased probability surviving and reproducing. As a result of this evolutionary advantage of the genetic variant, and the associated phenotype, the frequency of the mutation did increase and the phenotype became dominant. It is well known that Neolithization resulted also in other adaptive changes in genes, as for instance those associated with the ability to digest lactose and skin depigmentation [47–49]. Some studies focused on the ability to digest lactose contained in milk, demonstrated a positive selection on the lactase gene that occurred after the Neolithization period in Europe. These data supports the hypothesis that nutrients provided by dairy

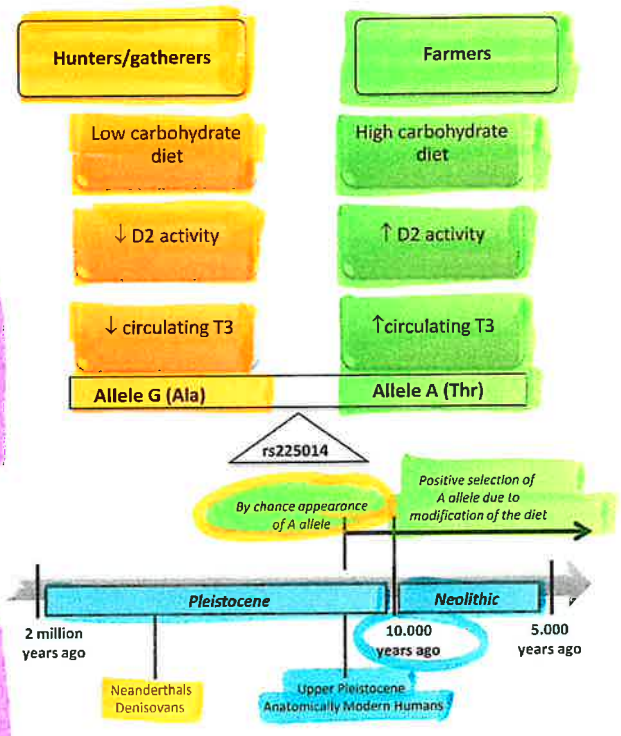



**Fig. 2** Schematic representation of rs225014 role in human evolution from Upper Pleistocene to nowadays. Neanderthals and Denisovans, who were hunters/gatherers with low carbohydrate diet, displayed G allele corresponding to p.92Ala amino acid linked to a reduced production of T3 hormone. Among Modern Humans, we assist to the by chance appearance of A allele corresponding to p.92Thr amino acid related to an increased production of T3. The transition to Neolithic food producers, associated with a high carbohydrate diet, may have contributed to the positive selection of A allele, which is, at present, the most widespread globally

were important for survival and further development in the recent history of Europe and other regions of the world [50]. Other authors provide direct evidence that strong selection favoring lighter skin, hair, and eye pigmentation has been operating since the Neolithic spread to higher latitudes [51]: the need to admit UVB radiation to catalyze the synthesis of vitamin D3, together with the decreased danger of folate photolysis at higher latitudes, may account for the observed skin depigmentation from prehistoric to modern times in this regions [47–52].

It is worth emphasizing that the Thr at position 92 seems to be specific for Anatomically Modern Humans, since it is not present in other mammalian species. These data suggest the possibility that this variant has been positively selected under the particular living conditions and diet habits.

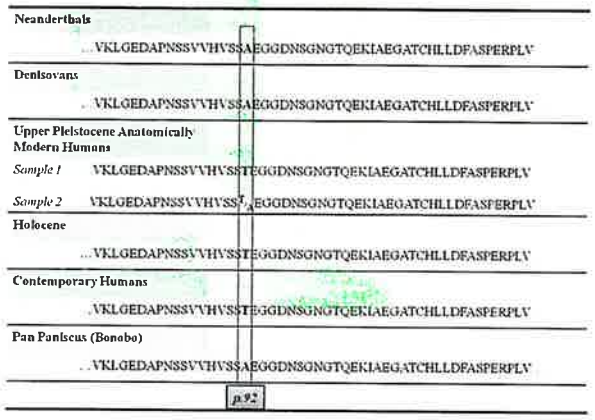
The authors are well aware of the fact that the low numbers of fossil individuals included limit the power of this study. In addition, since many of the fossils were found at the same sites, they might originate from related individuals and thus, they might share common ancestors and similar

**Table 1** (continued)

26 more specific populations, and allele and genotype frequencies are reported

B: data obtained from Genome Aggregation Database (GnomAD): allele frequencies for different populations are reported; genetic information is obtained from exome (left) and genome (right) data

**Table 2** Results of protein sequence alignment




Protein sequence alignment in the samples analyzed in this paper in comparison with contemporary humans. Representative sequences are reported

underwent thyroidectomy, this causes decreased levels of circulating T3 [7–12].

On the bases of our DNA analyses, one can assume that the A allele, which encodes for the Threonine, appeared for the first time during human evolution in Anatomically Modern Humans during the Upper Pleistocene. In this time period, there is the evidence of the presence of homozygous Thr/Thr and heterozygous Thr/Ala individuals (Table 2).

Nowadays, the presence of the p.Thr92Ala variant is not detrimental to health, but it may become harmful in particular conditions, such as thyroidectomy, in which the production of T3 at peripheral level is essential, and its decrease may be noxious [7–12], since the thyroid gland cannot compensate for the D2 deficiency. We can find a similar condition in presence of iodine deficiency: even then, all the mechanisms involved in T3 production become fundamental [37, 38].

It is supposed that Neanderthals often lived in regions where glaciations had depleted the soil of iodine [39]. In addition, the dominant presence of the p.Thr92Ala variant was likely to limit the peripheral T3 levels. This combination of genetic and environmental conditions provokes the question of whether or not Neanderthals in general were hypothyroidic. According to out recent knowledge, their diet was mainly based on dark meat with limited intake of fruit and vegetables [40, 41]. This animal-based diet was probably sufficient to provide the necessary intake of iodine and made them more tolerant than modern humans to the fluctuations

**Table 3** Amino acid at position 92 of D2 protein in mammalian species

| Amino acid | Species |
|---|---|
| ***T*** | ***Anatomically Modern Humans*** |
| ***A*** | ***Neanderthals*** |
| ***A*** | ***Denisovans*** |
| ***A*** | ***Pan paniscus*** |
| **A** | ***Pan troglodytes*** |
| **A** | ***Pongo abelii*** |
| **A** | ***Macaca fascicularis*** |
| **A** | ***Rhinopithecus roxellana*** |
| **A** | ***Rhinopithecus bieti*** |
| **A** | ***Cebus capucinus imitator*** |
| **A** | ***Gorilla gorilla gorilla*** |
| **A** | ***Callithrix jacchus*** |
| **A** | ***Macaca mulatta*** |
| **A** | ***Nomascus leucogenys*** |
| **A** | ***Saimiri boliviensis boliviensis*** |
| **A** | ***Macaca nemestrina*** |
| **A** | ***Papio anubis*** |
| **A** | ***Hylobates moloch*** |
| **A** | ***Piliocolobus tephrosceles*** |
| **A** | ***Chlorocebus sabaeus*** |
| **A** | ***Colobus angolensis palliatus*** |
| **A** | Enhydra lutris kenyoni |
| **A** | Mustela erminea |
| **A** | Mustela putorius furo |
| **S** | Vulpes vulpes |
| **S** | Canis lupus familiaris |
| **S** | Felis catus |
| **S** | Orycteropus afer afer |
| **S** | Trichechus manatus latirostris |
| **P** | Marmota marmota marmota |
| **P** | Sus scrofa |
| **P** | Pteropus vampyrus |
| **P** | Equus caballus |
| **P** | Erinaceus europaeus |
| **L** | Tupaia chinensis |
| **N** | Eptesicus fuscus |
| **R** | Manis javanica |

Primates are indicate in bold in the upper part of the table

*T* threonine, *A* alanine, *S* serine, *P* proline, *L* leucine, *R* arginine, *N* asparagine

genetic backgrounds. This is a common problem since sequence data of extinct human populations are limited and have often be obtained from small groups of individuals. Thus, this data may be not sufficient for high power SNP analysis. Moreover, in some cases the sequence data are difficult to find in the databases. Thus, for the future, it would be of fundamental importance to improve DNA data sharing and database accessibility, to make all the sequences available to the scientific community. Combining a better data accessibility with future analyses of the genomes and/or transcriptomes of additional Neanderthals, Denisovans and later Anatomically Modern Human fossils would make it possible to draw more clear-cut conclusions. Such conclusions are clearly needed to understand human evolution in more detail. It is of common interest to explore how our ancient relatives lived, how they evolved and why some of them extincted but others survived. Such knowledge may help us to explore current biological problems that impact todays every day life. Moreover, this knowledge might help us to predict how our life will continue in the future and how we will evolve.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Human and animal rights** This article does not contain any studies with human participants or animals performed by any of the authors.

**Informed consent** For this type of study, formal consent is not required.

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