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HED joins the growing list of inherited skin disorders caused by mutant members of the connexin family of proteins. A mutation (D66H) in *GJB2* was detected in Vohwinkel syndrome[13] and a missense mutation (G12R) in *GJB3* was observed in erythrokeratodermia variabilis[14] (EKV). The glycine residues at positions 11 and 12 of CX-30 and in CX-31, respectively, are conserved and lie in a stretch of highly conserved residues located in the cytoplasmic amino-terminal domain (Fig. 2*e*). The introduction of a positively charged arginine in the N-terminal domain or the mutation A88V, introducing a highly hydrophobic residue in the transmembrane M2 domain (Fig. 2*e*), may change the polarity of connexin channels and affect communication between cells. The *GJB6* mutations might also cause HED through haploinsufficiency of gap-junction channels, or by dominant-negative effect on normal CX-30 activity.

The central question raised by our data is the involvement of CX-30 in two different pathologies: non-syndromic autosomal dominant deafness, caused by a T5M mutation[9], and HED, here shown to be caused by different mutations of the same gene. In this respect, the different mutations in *GJB6* are comparable to those occurring in other genes encoding members of the connexin family. Mutations in *GJB3* are responsible for both EKV (ref. 14) and an autosomal dominant hearing impairment[15]. It is possible that the mutations detected in HED patients affect an epidermal-specific connexin function, and it may be that the multiple connexins expressed in keratinocytes do not compensate for the effect of the mutant CX-30. On the other hand, other connexins in auditory cells may be able to compensate for this effect. Additional studies are needed to understand how mutant connexins affect hearing cells in deafness or epidermis, nails and hair in HED.

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A view of Neandertal genetic diversity

The retrieval of mitochondrial DNA (mtDNA) sequences from the Neandertal type specimen from Feldhofer Cave in western Germany[1,2] made possible a comparison of DNA sequences from an extinct hominid with those from modern humans. Recently, a second mtDNA sequence from a Neandertal child found in Mezmaiskaya Cave in the northern Caucasus was determined and found to be similar to the type specimen[3]. To further study the Neandertal mtDNA gene pool, we analysed the amino acid composition and extent of amino acid racemization in 15 Neandertal bones found in the G3 layer[4,5] in Vindija Cave, Croatia.

Seven samples proved to have a high content of amino acids, an amino acid composition similar to that of contemporary bone, and a low level of racemization of aspartic acid, alanine and leucine[6], all features compatible with DNA preservation[7]. We dated one of the samples (Vi-75-G3/h-203; ref. 5) by accelerator mass spectroscopy to over 42,000 years before present (Ua-13873) and used it for five DNA extractions. In three of the extractions, we included N-phenacylthiazolium bromide (PTB), a compound that has been shown to improve DNA retrieval from late Pleistocene coprolites, probably due to its ability to cleave sugar-derived condensation products in which DNA may be entrapped[8].

We amplified multiple overlapping mtDNA fragments by PCR from the extracts, cloned the products and sequenced the insertions of multiple clones. When using primers that allowed both modern

Table 1 • Mitochondrial DNA sequence variation among three Neandertals, humans, chimpanzees and gorillas

Population	Individuals	Mean	Minimum	Maximum	s.d.
Neandertals	3	3.73	–	–	–
Humans	5,530	3.43	0.00	10.16	1.21
Europeans	1,433	2.21	0.00	7.61	0.92
Africans	919	3.91	0.00	8.52	1.16
Asians	1,633	3.03	0.00	9.63	0.98
Native Americans	1,388	3.06	0.00	9.66	1.05
Oceanians	157	3.80	0.00	9.62	1.14
Chimpanzees	359	14.81	0.00	29.06	5.70
Gorillas	28	18.57	0.40	28.79	5.26

The variation is expressed as the percentage of sequence positions that has changed in trees relating three mtDNA sequences.

human and Neandertal DNA sequences (as determined from the type specimen) to be amplified, we often observed clones similar to the Neandertal mtDNA sequences as well as clones similar to contemporary human mtDNA sequences. In those cases, the former class of sequences was deemed to be endogenous to the fossil. In seven amplifications performed from extractions in which PTB had been used, we found 27 clones carrying Neandertal sequences and 64 carrying contemporary human sequences, whereas in two amplifications from extractions in which no PTB had been used, all 20 clones sequenced carried human DNA sequences. Thus, PTB seems to specifically facilitate the retrieval of ancient DNA also from bones.

Following an established strategy[1], 357 bp of the hypervariable region (HVR)-1 and 288 bp of the HVR-2 were reconstructed from the Vindija 75 fossil (Fig. A, see http://genetics.nature.com/supplementary_info/). When compared with the type specimen sequence, they differed by nine substitutions and in the length of a stretch of cytosine and thymide residues in HVR-2. The sequences from the two Neandertals differ from those of 663 modern humans sampled from all areas of the world by 34.9±2.4 substitutions and by an insertion of an adenosine residue shared by the two Neandertals. They are not closer to 472 contemporary mtDNAs in Europe (35.3±2.1, range 29–43), the area where they existed until approximately 30,000 years ago, than to, for example, 151 African (33.9±2.8, range 28–42) or 41 Asian mtDNAs (33.5±2.1, range 29–38). This is reflected in a gene tree, where the two Neandertals group together to the exclusion of all modern humans (Fig. 1*a*). These results do not exclude that interbreeding between Neandertals and modern humans may have taken place[9], but they show that even if it occurred, Neandertals did not end up contributing mtDNA to the contemporary human gene pool.

The recent determination of 345 bp of the HVR-1 region from a Neandertal from Mezmaiskaya Cave[3] makes it possible to estimate the genetic diversity among three Neandertals. Although this represents a very small sample size, it is worth noting that the probability of sampling the deepest genetic divergence in a randomly mating population is n–1/n+1, where n is the number of sampled individuals[10]. In principle, the three Neandertal sequences therefore allow a probability of 50% to sample the deepest divergence among Neandertals. In fact, this is likely to be a conservative estimate because the samples are highly dispersed both geographically and temporally. A distance-based tree relating the three sequences was estimated, and the percent of positions that have changed in this tree taken as a measure of Neandertal mtDNA diversity. To compare this with modern humans, we estimated trees relating a total of 50,000 triplets of sequences randomly chosen among DNA sequences determined from 5,530 modern humans available in the database[11]. The same analysis was then performed for 359 common chimpanzees and 28 gorillas (Fig. 1*b*–*d* and Table 1). The diversity of the three Neandertal mtDNAs (3.73%) was lower than that of chimpanzees (14.82±5.70%) and gorillas (18.57±5.26%) and similar to that of modern humans worldwide (3.43±1.22%). When the human sequences were sorted into continental groups, the diversity among the three Neandertals fell within one standard deviation of the variation for Africans, Asians, Native Americans, and aboriginal Australians and Oceanians, whereas Europeans, which inhabit approximately the same geographical area as did the Neandertals, were less diverse than the Neandertals.

Although more extensive sampling of Neandertals is obviously desirable, the current sequences indicate that the diversity of Neandertals was restricted. Thus, it is highly unlikely that a Neandertal mtDNA lineage will be found that is sufficiently divergent to represent an ancestral lineage of modern European mtDNAs. Neandertals, however, seem to have been more similar to modern humans than to apes in having a low species-wide mtDNA diversity. In the case of humans, the low genetic diversity seen in both mtDNA and nuclear DNA sequences is likely to be the result of a rapid population expansion from a population of small size[12], often assumed to have been made possible by some cultural or genetic innovation, such as use of a complex language. If the Neandertals, similar to humans, had a diversity lower

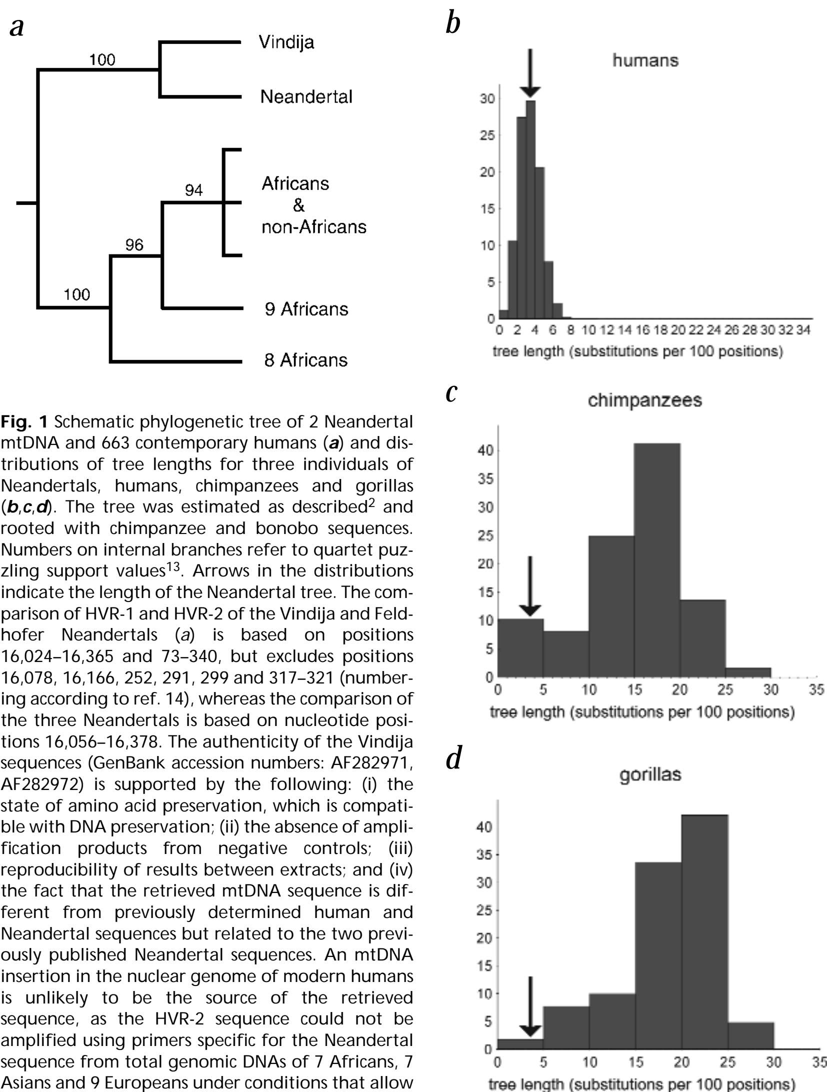

Fig. 1 Schematic phylogenetic tree of 2 Neandertal mtDNA and 663 contemporary humans (***a***) and distributions of tree lengths for three individuals of Neandertals, humans, chimpanzees and gorillas (***b***,***c***,***d***). The tree was estimated as described[2] and rooted with chimpanzee and bonobo sequences. Numbers on internal branches refer to quartet puzzling support values[13]. Arrows in the distributions indicate the length of the Neandertal tree. The comparison of HVR-1 and HVR-2 of the Vindija and Feldhofer Neandertals (*a*) is based on positions 16,024–16,365 and 73–340, but excludes positions 16,078, 16,166, 252, 291, 299 and 317–321 (numbering according to ref. 14), whereas the comparison of the three Neandertals is based on nucleotide positions 16,056–16,378. The authenticity of the Vindija sequences (GenBank accession numbers: AF282971, AF282972) is supported by the following: (i) the state of amino acid preservation, which is compatible with DNA preservation; (ii) the absence of amplification products from negative controls; (iii) reproducibility of results between extracts; and (iv) the fact that the retrieved mtDNA sequence is different from previously determined human and Neandertal sequences but related to the two previously published Neandertal sequences. An mtDNA insertion in the nuclear genome of modern humans is unlikely to be the source of the retrieved sequence, as the HVR-2 sequence could not be amplified using primers specific for the Neandertal sequence from total genomic DNAs of 7 Africans, 7 Asians and 9 Europeans under conditions that allow amplifications from less than a single copy per genome (data not shown). The type specimen HVR-1 has previously been similarly tested.

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than that of the great apes, in spite of inhabiting a region much larger than the apes, this may indicate that they also had expanded from a small population. Analyses of further Neandertal individuals will reveal if a population history similar to that seen in humans underlies the reduced diversity in Neandertals.

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Mutations in *AXIN2* cause colorectal cancer with defective mismatch repair by activating β-catenin/TCF signalling

Colorectal cancer (CRC) with defective DNA mismatch repair (MMR) is associated with alterations in one of several DNA MMR genes[1]. The downstream functional consequences of such defects are unclear. Here we show that *AXIN2*, encoding a Wnt-signalling component, is mutated in 11 of 45 CRC with defective MMR. The mutations stabilize β-catenin and activate β-catenin/T-cell factor (TCF) signalling, indicating its role in CRC development by linking defective MMR to the adenomatous polyposis coli (APC) pathway.

We previously cloned the human homologue of mouse conductin[2] (AXIN2). Like its mouse counterpart[3], AXIN2 interacts with APC, GSK3β and β-catenin (data not shown), thus making it a potential mutational target for colorectal cancer. To screen for *AXIN2* mutations, we first determined its genomic structure (data not shown) and screened DNA from 105 CRC tumours by denaturing HPLC (DHPLC; ref. 4). Of these, 45 had defective MMR defined by the presence of tumour microsatellite instability (MSI–H) and an absence of expression of MSH2 or MLH1 proteins, whereas the remaining 60 were MMR proficient with normal expression of these two proteins and no, or a low frequency of, microsatellite instability[5] (MSS or MSI–L, respectively). We identified 11 frameshift mutations (six 1-bp insertions and five 1-bp deletions), each occurring in the 4 mononucleotide repeat sequences located in exon 7 (Table 1 and Fig. 1*a*). We detected these mutations only in CRC with defective MMR (11/45) and not in MMR-proficient tumours (0/60; $P<10^{-5}$, Fisher's exact test). Frameshift mutations in *AXIN2* thus appear to be specifically associated with defective MMR in CRC.

We analysed the pathological impact of these mutations by immunohistochemical staining of β-catenin. We observed accumulation of β-catenin in tumour cell nuclei in 10 of 11 CRC with *AXIN2* mutations (Table 1), but not in the matched normal tissues or in 3 MSI–H CRC without *AXIN2*, *APC* or *CTNNB1* mutations (data not shown). Immunoblot analysis of normal fibroblast cells transfected with mutant *AXIN2* further demonstrated the accumulation of β-catenin in the nuclei (Fig. 1*b*). Screening of the same group of CRC with defective MMR for *CTNNB1* or *APC* mutations, as described[6–8], identified five *CTNNB1* mutations (5/45) and four *APC* truncation mutations (4/28 from available DNA). None of these mutations were found in the tumours with *AXIN2* mutations, suggesting that the *AXIN2* mutations, and not mutations in *APC* or *CTNNB1*, alter the APC pathway and contribute to the development of CRC in these tumours.

We tested the functional importance of *AXIN2* mutations in the development of CRC with defective MMR in a TCF reporter assay[9]. Plasmids containing multi-

Table 1 • Molecular and biochemical analysis of *AXIN2* in CRC with defective MMR

Patient ID	Allele(s)	*AXIN2* mutation	Predicted stop codon	Expressed allele(s)	β-catenin staining*
1095 (T1)	Wt/mut	2083 del G	L688X	Wt/mut	1–2
1219 (T2)	–/mut	2084 ins G	E706X	–/mut	1
1556 (T3)	Wt/mut	2084 ins G	E706X	Wt/mut	1
2174 (T4)	Wt/mut	2084 ins G	E706X	Wt/mut	1
27931 (T5)	Wt/mut	2084 ins G	E706X	Wt/mut	2–3
28076 (T6)	Wt/mut	2112 del C	L688X	Wt/mut	1
28675 (T7)	Wt/mut	2084 ins G	E706X	Wt/mut	3
30190 (T8)	Wt/mut	2100 del C	L688X	Wt/mut	0
30242 (T9)	Wt/mut1 and mut2	2015 ins A 2084 ins G	E706X E706X	Wt/mut1 and mut2	3
32633 (T10)	–/mut	2083 del G	L688X	–/mut	1
33271 (T11)	Wt/mut	2083 del G	L688X	Wt/mut	2–3

del, deletion; ins, insertion; –, deleted; X, stop codon; *Immunohistochemical nuclear staining of β-catenin was scored as negative (0), weak (1), or moderate to intense (2–3). T1–T11 in parenthesis refers to lanes in Fig. 1*a*.