Original Article

Huntington disease-like 2: the first patient with apparent European ancestry


Huntington disease-like 2 (HDL2) is a rare autosomal dominant disorder of the nervous system, apparently indistinguishable from Huntington disease (HD). HDL2 is caused by the expansion above 40 CTG/CAG repeats, in a variably spliced exon of the *junctophilin-3* gene, on chromosome 16q24.3. All patients described so far have been of African ancestry. A clinical evaluation, including the Unified Huntington’s Disease Rating Scale, and brain Magnetic resonance imaging were achieved in a 48-year-old Brazilian man of apparent European extraction, and presenting a picture very suggestive of HD. Gene mutation analysis (HD, HDL1, HDL2, dentatorubralpallidoluysian atrophy and spinocerebellar ataxia 17) was performed. After exclusion of the HD mutation and other HDL disorders, we identified an expansion of 47 CTG/CAG at the HDL2 locus. To clarify the origin of the mutation and estimate the patient’s ancestry, we performed haplotype studies and used the insertion/deletion polymorphisms method. Despite the fact that this patient had an estimated likelihood of 97.4% of being of European ancestry, the haplotype containing the expanded allele has been found only in Africans. Thus, this is the first HDL2 case reported in a patient with an apparent European ancestry, although bearing an African HDL2 haplotype. This work stresses the importance of performing the diagnosis of HDL2 in HD-like patients of various ethnicities, and particularly in highly mixed populations.

Huntington disease-like 2 (HDL2) (OMIM 606438) is a newly described disorder of autosomal dominant inheritance, the symptoms of which may be undistinguishable from Huntington disease (HD) (OMIM 143100). HD is a disorder of the central nervous system, characterized by involuntary choreic movements, progressive motor impairment, behavioural anomalies and cognitive decline.

HDL2 belongs to a group of HDL disorders, also including HDL1 (OMIM 603218), an autosomal dominant disease caused by an extra octapeptide repeat in the prion protein gene (*PRNP*), on chromosome 20p12 (1); and HDL3 (OMIM 604802), an autosomal recessive, mapped to 4p15.3 (2). Furthermore, two dominant spinocerebellar ataxias (SCAs), dentatorubralpallidoluysian atrophy (DRPLA) and SCA17, may also have overlapping symptoms with HD, namely Parkinsonism, dementia, chorea and dystonia (3).

HDL2 has not yet been found among Europeans (4–7) or Asians (8). HDL2 is the result of an expanded CTG/CAG repeat, in the *junctophilin-3* gene (*JPH3*), on chromosome 16q24.3 (6). Normal individuals have 6–28 repeats, whereas the mutated gene in patients contains 40–59 repeats (8).

The pathogenic mechanism underlying HDL2 remains elusive. Different *JPH3* transcripts because of alternative splicing and different polyadenylation sites have been described (6). The CTG/CAG tract may be located in intron 1 of the first isoform described (9) or within an alternatively spliced exon (exon 2A), either untranslated or translated to a polyleucine or to a
polyalanine stretch (6). The JPH3 gene encodes for junctophilin-3, a protein predominantly expressed in the brain (9, 10). The function of JPH3 is related to the formation of the junctional membrane structure in excitable cells, by interacting with the plasma membrane, through its cytoplasmic/sarcoplasmic reticulum membrane and spanning the endoplasmic/sarcoplasmic reticulum membrane through its C-terminal hydrophobic region (10). The JPH3 knockout mouse shows impaired performance of motor-coordination tasks, likely because of abnormal neuronal signalling (11). Despite the above evidence, the involvement of the mutant JPH3 in HDL2 pathogenesis has not yet been fully clarified. By contrast, a recent study showed that the CUG repeat-containing RNA foci are sufficient to cause cell toxicity in HDL2 (12).

In this work, we describe the first Brazilian HDL2 family. Given that the Brazilian population was originally formed from three ancestral roots (Amerindians, Europeans and Africans), we decided to study the patient’s ancestry. We have also performed a haplotype analysis to try to determine the origin of the mutated allele.

### Subjects and methods

**Subject**

A 48-year-old male was the fourth child of non-consanguineous parents. The family, which was of Brazilian origin, stated that they were not aware of any African ancestry, even many generations before.

His father, already deceased, had a similar clinical picture since age 50 years, suggesting an autosomal dominant transmission in this family. All his five sibs were asymptomatic; he had three asymptomatic offspring (aged 26, 24 and 22 years). The patient was neurologically examined, and a blood sample was obtained after informed consent. After the initial evaluation, he suffered a cerebral vascular accident, with secondary infections, and died in a septic state, without further neurological evaluation.

**Mutation analysis**

Genomic DNA was extracted from blood lymphocytes by standard methods. Repeat expansions causing HD, as well as other related disorders (HDL2, DRPLA and SCA17), were screened in the proband. Trinucleotide repeats in the HD, JPH3, ATN1, and TBP genes were amplified using primers and cycling conditions previously published (6, 13–15), with modifications to permit automated fluorescent genotyping. Forward primers were labelled at its 5’ end, with the fluorochromes 6-FAM or HEX. Polymerase chain reaction (PCR) was performed in a final volume of 25 µl, containing Taq buffer 1× (Applied Biosystems, Foster City, CA), 200 µM deoxyribonucleotide triphosphate (dNTPs), 1 mM MgCl2, 400 nM each primer, 1.5 U Taq Gold (Applied Biosystems) and 10% dimethyl sulfoxide (DMSO). Fragment separation was made using capillary electrophoresis in an ABI PRISM 310 Genetic Analyser; 1 µl of each PCR was mixed with 12 µl of deionized formamide and 0.5 µl GeneScan Rox 500 labelled marker (Applied Biosystems). PCR products were denatured at 95°C for 2 min, prior to the run. The presence of the eight extra octapeptide repeats on PRNP gene was analysed by PCR, followed by an agarose gel (16).

**Haplotype study: DNA cloning and sequencing**

We analysed the HDL2 repetitive tract and a downstream region of ~6.2 kb, using primers L237-2 5’-agatgcacgcgatcgg-3’ and JPH3R 5’-ggcatggcagtgaccagattg-3’. From the HapMap database (www.hapmap.org), we selected four single nucleotide polymorphisms (SNPs) encompassed within the analysed region: rs2562059, rs1864151, rs1864152 and rs2573124 (chromosome positions 86196356, 86199494, 86199574 and 86201418). These SNPs are located 920, 4059, 4140 and 5985 bp away from the end of the CTG segment. In accordance to the HapMap database, these are the best SNPs in the region taking into account their power of discrimination between European and African populations. The rs2562059 alleles C/G have a relative frequency of 0.6/0.4 in Europeans and 0.267/0.733 in Africans; the rs1864151 alleles A/C have frequencies of 0.617/0.383 in Europeans and 0.525/0.475 in Africans; the rs1864152 alleles A/C have frequencies of 0.158/0.842 in Europeans and 0.208/0.792 in Africans; and the rs2573124 alleles C/A have frequencies of 0.602/0.398 in Europeans and 0.267/0.733 in Africans.

The PCR assay was performed in a 50 µl final volume of 1× Expand High Fidelity Buffer (Roche Applied Science, Mannheim, Germany), 200 µM dNTPs, 300 nM of each primer, 10% DMSO, 125 ng of genomic DNA and 2.6 U of Expand High Fidelity enzyme mix (Roche). Cycling conditions were performed according to the manufacturer’s instructions (Expand High Fidelity PCR System). DNA fragments were

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purified using a kit (Amersham Biosciences, Buckinghamshire, UK) after excision of DNA agarose bands.

By cloning the purified DNA fragments (~6.2 kb) into the pCR4-TOPO vector (Invitrogen, Carlsbad, CA), under the manufacturer’s instructions, we obtained isolated bacterial colonies, carrying either the normal or the expanded allele. Sequence analysis was performed by cycle sequencing in an ABI PRISM 310 using T3 and T7 primers (to confirm insert orientation), as well as primer L237-2 (to confirm the CTG repeat size); primers SNP3JPH3F 5’-agacctgtctccagaggtg-3’ and SNP3JPH3R 5’-tctgtctgcagtggtttggtt-3’ (to detect SNP rs2562059); primers SNP4JPH3F 5’-ctctgaatatgacgagctctc-3’ and SNP5JPH3R 5’-actcagttcgacgacc-3’ (to detect SNPs rs1864151 and rs1864152); and primers JPH3R and SNP6JPH3F 5’-gcttactcactcgccactcacctc-3’ (to detect SNP rs2573124).

Patient’s ancestry by the INDELS’ method

The patient was typed for a set of 40 biallelic short insertion/deletion polymorphisms (INDELS). As ancestral population references, previous data from the Human Genome Diversity Cell Line Panel (HGDP-CEPH) was used on 161 Europeans, 126 Africans and 103 Amerindians (17). To estimate the ancestral contribution from these three populations, we used the STRUCTURE program version 2.1 (18) (available at http://pritch.bsd.uchicago.edu/software.html). This software uses multilocal genotypes to allocate ancestry proportions of individuals to different clusters (populations). The software defines k clusters (where k is provided by the user), each of them being characterized by a set of allelic frequencies for each locus.

Results

Clinical features

The neurological examination of this 48-year-old patient was consistent with the diagnosis of HD. The Unified Huntington’s Disease Rating Scale motor section (0 = normal and 120 = maximal severity) was applied; the patient’s score was 49. The patient turned listless, introspective, and with delayed response by the age of 44 years. This was followed by slurring speech, difficulties in walking, progressive deterioration of mental abilities, and choreiform movements. On physical examination, he was disoriented in time and space, and recent memory was lost. His gait was a widebased, slow and interrupted by choreic movements of his legs and trunk. There was dysarthria, motor impersistence, dysmetria, and dysdiadochokinesia; his ocular pursuit was interrupted. Deep tendon reflexes were normal. Chorea was important in the tongue, trunk, upper and lower limbs, and mild-to-moderate rigidity was present in his arms.

Brain magnetic resonance imaging

The fluid attenuation inversion recovery, T1- and T2-weighted images showed signs of brain atrophy, marked enlargement of the ventricles, mild periventricular white matter lesions, and bilateral atrophy of the caudate nucleus (Fig. 1).

Gene analysis

The genetic study of the HD locus showed a normal allele with 23 CAGs and a large normal allele with 30 CAGs, excluding the clinical diagnosis of HD. Repeat expansions in the TBP (37/38 CAA/CAG) and ATN1 (13/13 CAGs) genes were also excluded; we also could not find the octapeptide PRNP expansion of 192 bp, characteristic of HDL1. Finally, we tested for HDL2 and detected a CTG/CAG repeat expansion with 47 CTGs and a normal allele of 14 CTGs in the JPH3 gene.

Haplotype study

Sequence analysis of ~6.2 kb downstream from the CTG segment showed the presence of haplotypes (CTG)$_{14}$-CACC and (CTG)$_{47}$-GACA. Comparing these to haplotype frequencies published in the HapMap database, we could conclude that the block CACC is much more frequent in Europeans (42.5%) than in Africans (1.7%); but, the haplotype with the expanded allele GACA has, thus far, been found only in persons of African ancestry (16.3%). In addition, the four intragenic SNPs (GACA) found within the mutant allele corresponded to ancestral alleles according to the SNPs database (www.ncbi.nlm.nih.gov/SNP).

Patient’s ancestry

The patient and his relatives are of Brazilian origin. They did not refer any known African ancestor. In view of previous cases of HDL2 having been described exclusively in persons of definite
or possible African origin, we tried to gain further insight into the patient’s ancestry. After typing the 40-indel set, the software estimated a proportion of European, Amerindian and African ancestry of 97.4% (95% CI, 74.3–100.0%), 1.9% (95% CI, 0.0–23.4%) and 0.7% (95% CI, 0.0–9.6%).

**Discussion**

We identified the first patient with HDL2 reportedly of European ancestry, thus presenting evidence that the *JPH3* mutation may also be found in patients without apparent African origin.

The clinical presentation of this 48-year-old Brazilian male included choreic movements, rigidity, dysarthria, dementia and behavioural anomalies, as observed before in other HDL2 patients. Magnetic resonance imaging showed signs of brain atrophy, marked enlargement of the ventricles, mild periventricular white matter lesions and bilateral atrophy of the caudate nucleus, indistinguishable from those observed in HD. A genetic test excluded an *HD* expansion. At our laboratory, only about 4% of cases clinically diagnosed as HD do not carry the *HD* mutation (19). Other groups reported HDL cases, in their series, to be between 1% (13) and 7% (5). After exclusion of other HDL disorders, we eventually found a 47 CTG/CAG repeat expansion at the *JPH3* gene; affected individuals usually have 40–59 CTG/CAG repeats (6, 8).

The expanded allele had most probably been inherited from his father, who died with similar symptoms, without being genetically tested. Age of onset was 44 years in agreement with the correlation with repeat length of HDL2 (8).

Twenty-five HDL2 families described to this date had definite or possible African ancestry (12). The pedigree in which the mutation was first detected was of African-American origin (6, 20). Then, other African-American families were described (8, 21, 22): a Moroccan woman, from an area predominantly populated by individuals

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**Fig. 1.** Brain magnetic resonance imaging of the patient. Coronal T2-weighted image shows severe caudate head atrophy (a); axial T2-weighted images show diffuse basal ganglia atrophy, white matter lesions and brain atrophy (b, c and d).
of sub-Saharan origin, was found in an European collection (5); the Mexican pedigree (8, 22) originated from a region colonized by Africans; in an European group of 252 HDL patients, one HDL2 case was a black person from the French West Indies (23); and at least 15 families were from South Africa, where the prevalence of HDL2 has been described to be higher than in any other country (24). HDL2 accounts only for 1% of the HDL patients in North America and has not been found in Japan (8). Thus, it has been proposed that the diagnosis of HDL2 should be considered preferentially in persons of African origin. The higher frequency of the HDL2 mutation in African people could be explained by a common ancestor or a tendency for further expansion in a specific genetic background. Very recently, the first person with HDL2 and middle-eastern origin (from Kuwait) was described, suggesting a possible second founder event (25); however, the African ancestry of this patient was not completely excluded.

In our patient, the haplotype carrying the normal allele (CTG)14-CACC is much more frequent in Europeans (42.5%) than in Africans (1.7%); nevertheless, the haplotype with the expanded allele (CTG)97-(GACA) has only been found in persons of African ancestry (16.3%). Because our patient was Brazilian, it was difficult to determine the exact family ancestry. The patient and his relatives, however, considered themselves as Caucasians and did not refer any African ancestor. Confirming this assumption, the INDELS method showed that the likelihood of his ancestry was 97.4% European, 1.8% Amerindian and 0.7% African. There is, however, an important caveat: these estimates were based on a sample of only 40 loci, and the 95% confidence limits for African ancestry extended from 0% to 9.6%. The fact that he carries a haplotype exclusively described in persons of African origin shows, however, he should have at least one African ancestor.

This case shows that the diagnosis of HDL2 should always be considered, whenever HD has been excluded and the clinical presentation may suggest it, regardless of the apparent origin of the family, and particularly so in populations of mixed ancestry. It would be interesting now to compare ancestral haplotypes among families with HDL2.

Acknowledgements

The authors wish to thank Paula Magalhães for her technical support. We also wish to thank Maria do Carmo Costa and Sandra Martins for their valuable comments. Cláudia Santos is a recipient of a scholarship from IBMC – Instituto de Biologia Molecular e Celular.

References


HDL2 in a patient of apparent European ancestry