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APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy

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We report duplication of the *APP* locus on chromosome 21 in five families with autosomal dominant early-onset Alzheimer disease (ADEOAD) and cerebral amyloid angiopathy (CAA). Among these families, the duplicated segments had a minimal size ranging from 0.58 to 6.37 Mb. Brains from individuals with *APP* duplication showed abundant parenchymal and vascular deposits of amyloid-β peptides. Duplication of the *APP* locus, resulting in accumulation of amyloid-β peptides, causes ADEOAD with CAA.

Missense mutations in the genes encoding amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*) or presenilin 2 (*PSEN2*) cause autosomal dominant early-onset Alzheimer disease (ADEOAD) or, more rarely, hereditary dementia with cerebral amyloid angiopathy (CAA) (for review see ref. 1). Because Alzheimer disease associated with CAA is also found in Down syndrome[2], we reasoned that the *APP* locus located on chromosome 21q21 might be affected by gene dosage alterations in a subset of demented individuals. To test our hypothesis, we analyzed *APP* using quantitative multiplex PCR of short fluorescent fragments (QMPSF), a sensitive method for detecting duplications that is based on the simultaneous amplification of multiple short genomic sequences using dye-labeled primers under quantitative conditions[3] (**Supplementary Methods** online). This analysis was performed in 12 unrelated individuals with ADEOAD in whom a previous mutation screen of *PSEN1*, *PSEN2* and *APP* had been negative[4], 70 unrelated individuals with familial late-onset Alzheimer disease and 100 healthy control subjects. Five of the individuals with ADEOAD belonged to Alzheimer disease–affected families in which the co-occurrence of CAA had been diagnosed according to neuropathological[2] or clinical criteria (intracerebral hemorrhages (ICH) in at least one affected individual). In the five ADEOAD-CAA index cases, we found evidence for a duplication of the *APP* locus (**Fig. 1a**). In the corresponding families, we observed that the *APP* locus duplication was present in affected subjects but not in healthy subjects over the age of 60 years (**Fig. 1b**).

Subsequent QMPSF analyses targeting the genes marked by vertical bars in **Figure 1c** revealed heterogeneity of the duplication among the five families. The duplicated segments had a minimal size ranging from 0.58 to 6.37 Mb and contained from 5 to 12 annotated genes, centromeric to the Down syndrome critical region[5]. Recent surveys of structural polymorphism of the human genome have not identified any duplication involving the *APP* locus[6], which further supports the view that these duplications are disease related. In families F009 and F229, a low-copy repeat associated with both intra- and interchromosomal rearrangements[7] was located in the interval defined by QMPSF for the telomeric boundaries of these duplications. FISH performed on peripheral blood lymphocytes from two affected subjects belonging to families F037 and F229 showed three signals in interphase nuclei and, in all mitoses analyzed, a larger-sized signal on one copy of chromosome 21, consistent with a segmental duplication including the *APP* locus (**Fig. 1d,e**).

Finally, analysis by quantitative fluorescence PCR (QF-PCR) of the segregation of two microsatellite markers located within *APP* intron 1 and 330 kb centromeric from the *APP*, respectively, provided results fully consistent with those obtained by QMPSF analysis: (i) in each affected individual heterozygous for a given marker, we observed a twofold larger peak corresponding to one allele as compared to the peak seen for a control subject harboring the same alleles; (ii) different haplotypes were segregating with the disease among the five families, which is consistent with the finding that the size of the duplicated region differed between kindreds (**Supplementary Fig. 1** online).

The phenotypes of the affected subjects ($n = 19$) in the five families were similar. None had mental retardation before the onset of dementia. Retrospective examination of medical records and further clinical assessment[8] of the living affected subjects from families F037 and F229 did not reveal any clinical feature suggestive of Down syndrome. In all affected individuals, the most common clinical manifestation was progressive dementia of Alzheimer disease type (mean age of onset 52 ± 4.4 years) associated, in some cases (**Fig. 1b**), with lobar ICH.

Neuropathological examination of the brains of five individuals from three of the kindreds (F019, F028 and F037) showed abundant amyloid deposits, present both as dense-cored plaques and as diffuse deposits, in all regions analyzed. Neurofibrillary tangles were noted in the hippocampal cortex, the limbic system and the isocortex, and their topography and density were consistent with Braak stage V–VI[9]. Overall, these features were consistent with a diagnosis of definite Alzheimer disease according to the Consortium to Establish a Registry

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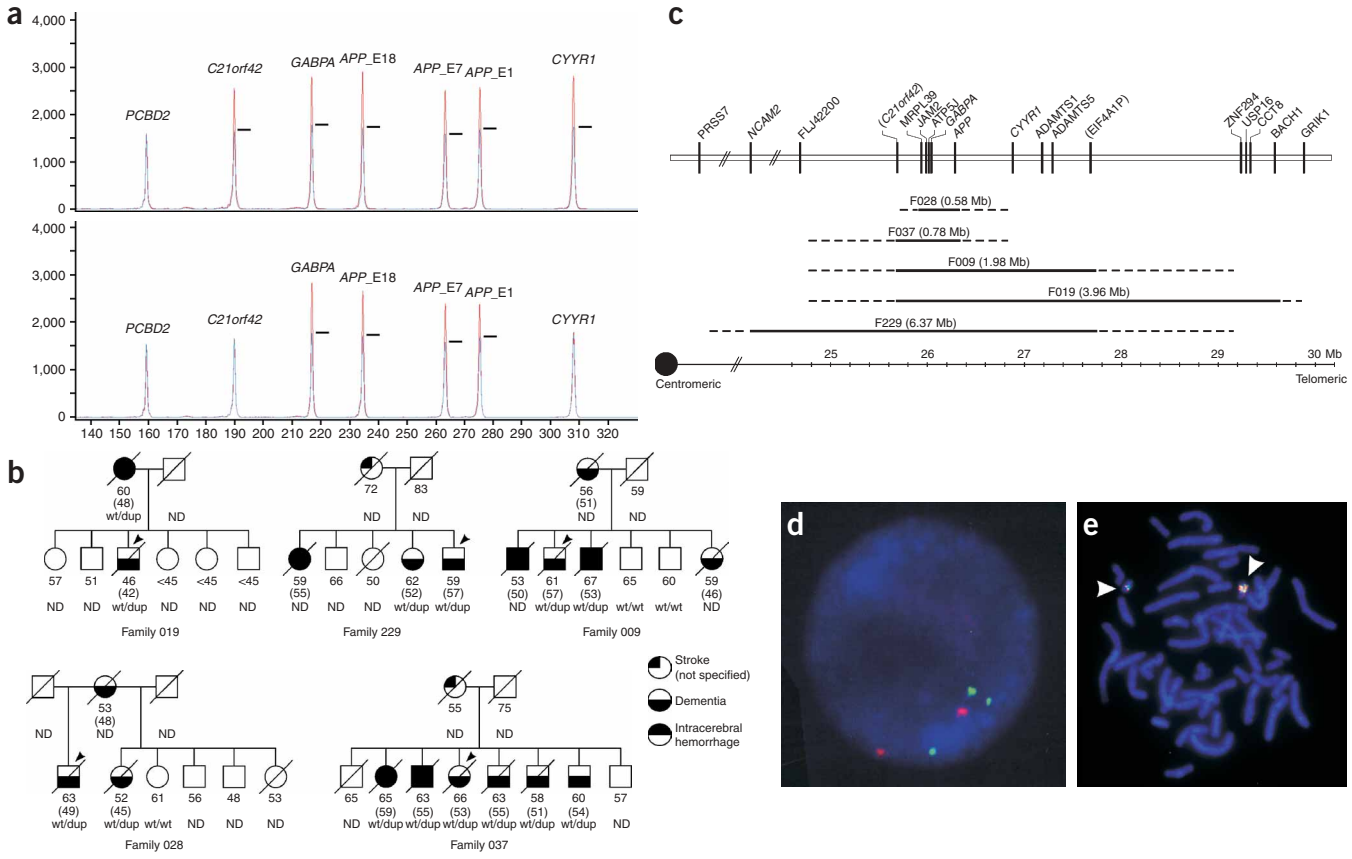

Figure 1 *APP* locus duplication in five kindreds. (**a**) Detection by QMPSF of *APP* duplications. The electropherogram of the affected subject (in red) was superimposed on that of a normal individual (in blue) by adjusting to the same level the peaks obtained from the control amplicon *PCBD2* located on chromosome 5. The vertical axis shows fluorescence in arbitrary units and the horizontal axis indicates the size of the amplicon in base pairs. Horizontal bars indicate heterozygous duplications of the amplicons, detected by a 1.5-fold heightening of the corresponding peaks. This QMPSF covers four genes located at 21q21: *C21orf42* (exon 2), *GABPA* (exon 8), *APP* (exons 1, 7 and 18) and *CYYR1* (exon 4). Above, patient with trisomy 21; below, index case of family F028 (*C21orf42* and *CYYR1* are not duplicated in this family). (**b**) Partial pedigrees of the five families. dup, duplication; ND, not determined; wt, wild type. Age at death or current age and age of disease onset (in parentheses) are indicated. Probands are identified by an arrow. (**c**) Gene content (excluding pseudogenes, ORFs and LOCs) of the duplicated regions in the five families. Vertical bars, genes analyzed by QMPSF; horizontal bars, minimal size of the duplicated regions; dotted lines, intervals of the duplication boundaries. (**d**,**e**) FISH analysis from peripheral blood lymphocytes of a patient from family F229. (**d**) Interphase cells. Hybridization signals were generated using simultaneously the BAC clone RP11-15D13 (green spot) and the LSI 21 probe (red spot), which hybridize at 21q21.3 and 21q22.13–q22.2, respectively. (**e**) Metaphase chromosomes. Hybridization signals were generated using simultaneously the BAC clones RP11-15D13 (green spot) and RP11-66H5 (red spot), both located at 21q21.3.

for Alzheimer's Disease (CERAD) criteria[10]. However, the most prominent feature was severe CAA[2]. In all regions analyzed, CAA was extensive, consisting of an acellular thickening of the leptomeningeal vessels as well as superficial and deep intraparenchymatous small arteries, capillaries and venules. Abundant circumferential amyloid deposits had invaded the arteriolar walls, extending over the adventitia, with densely packed fibrils radiating from the affected vessels into the surrounding neuropil. Myelin pallor or focal areas of demyelination were observed around the majority of vessels, sometimes containing iron-laden macrophages. In addition, in three brains, patchy small infarcts were noted in the cerebellar cortex, the pontine nuclei, the periaqueductal gray, the entorrhinal cortex and the hemispheric white matter, where microinfarcts were located at short distance from the affected vessels. Staining of lesions with antibodies specific for the amyloid-β (Aβ) peptides Aβ40 or Aβ42 showed that the vascular amyloid was predominantly composed of Aβ40, whereas parenchymal plaque cores contained Aβ42 and were surrounded by Aβ40 deposits (**Fig. 2**).

Quantification by ELISA of the abundance of Aβ, tau and phospho-tau in the cerebrospinal fluid (CSF) of one affected individual showed a typical Alzheimer disease–type pattern (namely, a decrease of Aβ42 and a slight increase of tau and phospho-tau). Notably, this pattern is also found in the CSF of individuals with Down syndrome who are developing dementia[11]. *APOE* genotype (ε3/ε3 in 11 subjects and ε3/ε4 in 3 subjects) did not influence the age of onset of dementia or the occurrence of ICH.

In Down syndrome, it is generally agreed that the widespread Aβ deposits consistently found in brains of affected individuals over 40 years of age result from the presence of an extra copy of *APP*. Indeed, this gene dosage alteration is responsible for a ≈1.5-fold increase in the abundance of *APP* mRNA level in trisomic brains[12], but the possible contribution of other genes located on chromosome 21 to the process of Aβ accumulation cannot formally be excluded. It has previously been shown that the proximal 21q region is critical for Aβ accumulation, as a partial trisomy involving the distal 21q region, outside of the *APP* locus, has been found not to be associated

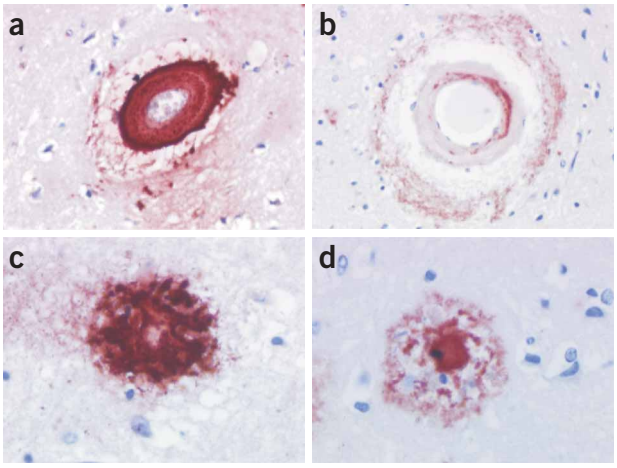

Figure 2 Immunochemical characterization of CAA and senile plaques in brain tissues of a patient from family F037. (**a**–**d**) Vascular amyloid (**a**,**b**) and senile plaques (**c**,**d**) were stained using antibodies labeling the Aβ40 (**a**,**c**) or Aβ42 peptide (**b**,**d**).

with Alzheimer disease–type neuropathology in a subject 78 years of age[13]. Our data provide compelling evidence that *APP* locus duplication causes ADEOAD associated with CAA and demonstrate that dosage alteration of a minimal chromosome 21 region, including *APP* and only four other genes (**Fig. 1c**), is sufficient to induce the formation of Aβ deposits.

In our whole ADEOAD cohort, which includes 65 families, the frequency of the *APP* locus duplication can be roughly estimated to be 8% (5 of 65), which corresponds to half the contribution of *APP* missense mutations to ADEOAD[4]. In 1987, it was claimed that *APP* locus duplication had been detected in three individuals of French origin with sporadic Alzheimer disease[14], but this finding has never been replicated. There is no relationship between our families and those three individuals with sporadic Alzheimer disease. In the present study, the genomic rearrangement was found in families in which Alzheimer disease and CAA coexist, implying that, in addition to rare *APP* or *PSEN* mutations associated with this particular phenotype (OMIM 104760, 104311 and 600759), *APP* locus duplication also causes this CAA variant of ADEOAD. Following the recent report of α-synuclein triplication in Parkinson disease[15], this provides further evidence that gene dosage alterations can be involved in the etiology of mendelian neurodegenerative disorders caused by protein or peptide accumulation.

Note: Supplementary information is available on the Nature Genetics website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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