both the HLH and LZ motifs which are protein-protein interaction domains (*2, 6, 8, 17*). Indeed, we demonstrated (Fig. 2) that mTFE3-L can form heteromultimers with mTFE3-S. Furthermore, the B-HLH protein MyoD and protein products of Achete-Scute are negatively modulated by formation of mixed multimers with HLH proteins Id and Extramacrochaete (*18*). However, the phenomenon we report is different because mTFE3 heteromultimers bind DNA, retain low activation capability, and are generated from the same gene.

Although many transcription factors appear to be controlled at the transcriptional level, regulation at other levels is necessary to avoid an endless progression of transcriptionally controlled events. Several transcriptional regulators encoded by alternatively spliced mRNAs have diverse phenotypes (*14, 15, 19–24*). Among these are the viral gene products E1A (*19*) and E2 (*20*), the homeobox gene product XIHbox-1 (*21*), c-Myb (*22*), Erb-A (*23*), and E2A (*24*). A trans-dominant effect has been noted for an alternative splice product of FosB that decreases full-length FosB activity twofold in cotransfection assays in which ratios of the two forms reflect physiological ratios (*14*). Differential splicing is emerging as an important posttranscriptional control point for the regulation of different aspects of transcription factor activity.

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26. Confluent plates of NIH 3T3 cells were passaged (1:10) 2 days before transfection by calcium phosphate coprecipitation as described (*25*). CAT activity was normalized for transfection efficiency by the cotransfection of an expression construct that contained the human growth hormone cDNA (Allegro, San Juan Capistrano, CA) in all transfections. Hormone secreted into the medium was quantitated by radioimmunoassay.
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A Mutation in the Amyloid Precursor Protein Associated with Hereditary Alzheimer's Disease

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Alzheimer's disease is a form of localized amyloidosis characterized by cerebral cortical amyloid plaques, neurofibrillary tangles, and amyloid deposits within the walls of leptomeningeal vessels. Although most cases of Alzheimer's disease are sporadic, kindreds with autosomal-dominant inheritance of the syndrome suggest that a single mutation may be important in pathogenesis. Direct sequencing of DNA from a family with autopsy-proven Alzheimer's disease revealed a single amino acid substitution (Phe for Val) in the transmembrane domain of the amyloid precursor protein. This mutation correlates with the presence of Alzheimer's disease in all patients in this study, and may be the inherited factor causing both amyloid fibril formation and dementia.

HEREDITARY OR FAMILIAL ALZHEIMER'S disease is an autosomal-dominant form of localized amyloidosis. Patients with hereditary Alzheimer's disease typically develop three pathologic lesions: (i) senile plaques in the cerebral cortex characterized by a central amyloid core surrounded by dystrophic neurites; (ii) neurofibrillary tangles; and (iii) congophilic angiopathy of the leptomeningeal vessels. The amyloid deposits in the senile plaques and in the blood vessel walls contain a fibril subunit protein of 39 to 43 amino acid residues (*1*), which is a portion of the carboxyl terminus of the amyloid precursor protein (APP). Sequence analysis of cDNA clones of APP has shown that there are multiple forms of mRNA which are the result of alternate splicing of a transcript from a single gene (*2–5*). Although the sequence of the APP gene from some patients with either sporadic or familial Alzheimer's disease is normal (*6–8*), a missense mutation in the membrane-spanning domain of the APP gene has been identified in patients from several families with familial Alzheimer's disease (*9, 10*).

We used genomic DNA from three generations of a family with classic early-onset autosomal-dominant Alzheimer's disease to determine whether the disease in this family is associated with a point mutation in the APP gene. We amplified and sequenced exon 15 of the APP gene using the polymerase chain reaction (PCR) (Fig. 1) (*7, 9, 11*).

Affected members of this family show clinical onset of disease with short-term

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Fig. 1. DNA sequence of region amplified by PCR (nucleotides 1732 through 2036). Lowercase letters designate introns; capital letters designate exon 15, which encodes the carboxyl-terminal portion of amyloid β protein, amino acids 614 through 669, (exon 17 if numbered by the APP770 transcript) (*5*). Solid lines indicate oligonucleotide primers used in the PCR reactions. The mutation at position 1924 is in the box. Solid arrowhead indicates the carboxyl terminus of the longest β-amyloid peptide sequence that has been reported (43 residues).

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ccaaatgtccccgtcatttaagaaatgaaattcttctaattgcgtttataaattgta
aattatattgcatttagaaattaaaattctttttcttaatttgttttcaag GTG TTC
                                                    Val Phe

TTT GCA GAA GAT GTG GGT TCA AAC AAA GGT GCA ATC ATT GGA CTC
Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu

                                        ▼
ATG GTG GGC GGT GTT GTC ATA GCG ACA GTG ATC GTC ATC ACC TTG
Met Val Gly Gly Val Val Ile Ala Thr Val Ile Val Ile Thr Leu
                                        T
                                        Val
                                        Phe

GTG ATG CTG AAG AAG AAA CAG TAC ACA TCC ATT CAT CAT GGT GTG
Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile His His Gly Val

GTG GAG gtaggtaaacttgactgcatgtttccaagtgggaattaagactatgagag
Val Glu
```

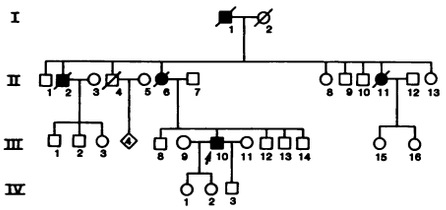

Fig. 2. Abbreviated pedigree of the family with early-onset Alzheimer's disease. Solid symbols indicate affected individuals. Tissue DNA was studied from all affected individuals in generation II. DNA isolated from peripheral blood leukocytes of III-10 and the five unaffected individuals in generation II was studied. Arrow denotes propositus.

memory problems in their 40s. Other cognitive difficulties develop as the disease gradually progresses (Fig. 2). Disease duration is typically 7 years. Postmortem examinations of the brains of three members of generation II showed histologic lesions typical of Alzheimer's disease with only minor vascular amyloid deposits and no evidence of cerebral hemorrhage (Fig. 3). Individuals II-2, II-6, and II-11 developed dementia at ages 41, 42, and 45, and died at ages 49, 48, and 53, respectively. One individual in generation III is presently 44 years of age and suffers from a severe presenile dementia. DNA from the five unaffected members of generation II, who are well beyond the age for expression of disease in this kindred, was also analyzed.

Genomic DNA was isolated from tissue specimens or peripheral blood leukocytes. Liver tissue of subject II-11 had been frozen at the time of autopsy in 1980. Brain tissue from subject II-6 had been fixed in formalin in 1968. For DNA extraction, the cerebellum was used because of the high density of cells. For subject II-2, DNA was extracted from a Congo red–stained histologic section of cerebral cortex (*12*).

Direct sequencing of PCR-amplified DNA of patient III-10 showed both a guanidine and thymine at position 1924 (Fig. 4) of APP. Thus, the individual was heterozygous with both a normal GTC (valine) and a variant TTC (phenylalanine) codon. Direct sequencing of amplified tissue DNA from the three affected members in generation II showed that each was heterozygous for the point mutation at position 1924. Direct DNA sequencing for the five unaffected generation II siblings who are beyond the usual age of onset of disease revealed only the normal guanine at position 1924. In addition, analysis of DNA from 100 unrelated individuals failed to show this mutation, which suggests this is not a polymorphism that co-segregates with disease by chance.

In contrast to linkage analyses that do not show association of Alzheimer's disease with the APP gene (*13, 14*), the occurrence of the mutation in the APP in individuals from two generations of a kindred affected with Alzheimer's disease is evidence for this mutation as the cause of amyloid deposition and dementia in this kindred. This mutation is consistent with observations of familial amyloidotic polyneuropathy (FAP), in which multiple single amino acid substitutions are associated with amyloid fibril formation (*15*). Although the pathogenesis of disease in systemic amyloidosis is generally considered to involve the formation of amyloid deposits that lead to destruction of normal tissue, similar conclusions cannot yet be drawn for hereditary Alzheimer's disease. The dementia may be in part the result of altered function of the variant APP protein and not the direct result of displacement of normal tissue by amyloid fibrils.

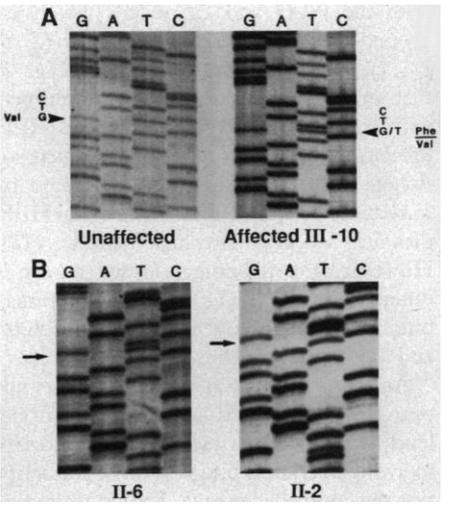

Fig. 4. Autoradiographs of sequencing gels of the APP gene exon 15 in affected and unaffected individuals (*22*). (**A**) Positive strand sequence of DNA from peripheral blood. (**B**) Sequences from formalin-fixed brain tissues. Sequencing of the opposite strand confirmed the mutation (*23*). Numbering is according to the APP695 transcript (*2*).

This amino acid substitution (Val to Phe) is at the same position in APP as the Val to Ile substitution found by Goate *et al.* in another family with early-onset hereditary Alzheimer's disease (*9*). This position is two residues beyond the carboxyl terminus of the β-amyloid peptide subunit isolated from fibrils. Because the carboxyl terminus of the β-amyloid peptide is heterogeneous and the amyloid protein from neither of the families found to have mutations at position 1924 has been studied, this amino acid may be included in the amyloid subunit protein in these particular kindreds. In any case, this position in the APP molecule must be important both in the generation of amyloid fibril deposits and Alzheimer's disease.

Single amino acid substitutions are also found in other forms of hereditary amyloidosis. In the transthyretin amyloidoses (FAP, types I and II), a number of single amino acid substitutions in transthyretin are

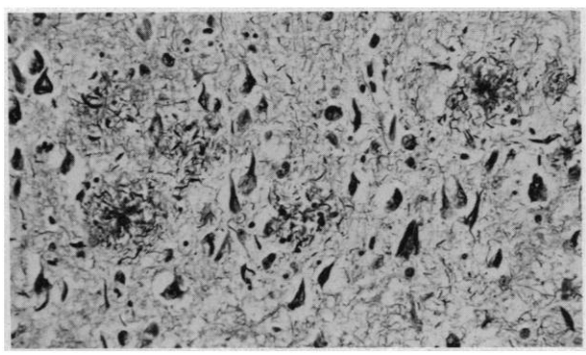

Fig. 3. Neurofibrillary tangles and senile (neuritic) plaques in the subiculum of II-11. In the plaques both a neuritic crown and a prominent amyloid core are seen. The majority of nerve cells contain neurofibrillary tangles. Preparation was stained with DeMyer modification of Hortega silver carbonate method; magnification, ×250.

associated with the amyloid syndrome (*15*). Similarly, FAP type III is associated with amyloid deposits that contain a peptide of apolipoprotein A1 with a single amino acid substitution (Gly to Arg) (*12*). In Gerstmann-Sträussler-Scheinker disease (GSS), a hereditary adult-onset dementia with cerebral amyloid plaques composed of a fragment of the prion protein, every GSS family studied to date has a mutation in the gene coding for this protein (*16–18*). Hereditary cerebral hemorrhage with amyloidosis in Icelandic kindreds (HCHWA-I) is associated with a single amino acid substitution in cystatin C (*19*), and a similar syndrome in Dutch kindreds (HCHWA-D) is associated with a single amino acid substitution of Gln for Glu in APP (*20*).

Although other single amino acid substitutions in APP may be associated with amyloid deposits and Alzheimer's disease, it is unlikely that single amino acid substitutions in APP can explain all sporadic forms of Alzheimer's disease. Recently it has been proposed that in some cases of FAP, normal transthyretin may form amyloid deposits in association with unknown aging factors (*21*). Similarly, in Alzheimer's disease, different pathogenic processes may lead to a common clinical presentation.

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23. J. Murrell *et al.*, unpublished results.

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Regulation of Transendothelial Neutrophil Migration by Endogenous Interleukin-8

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Movement of neutrophils from the bloodstream to inflamed tissue depends on the activation of both the neutrophil and the endothelial cell. Endothelial cells lining the postcapillary venule respond to proinflammatory mediators by expressing adhesion molecules and synthesizing a variety of neutrophil-activating factors. Endothelial cell production of a 77–amino acid variant of interleukin-8 (IL-8) was found to be a requirement for the invasion of neutrophils through a vessel wall model. IL-8 secreted by cytokine- or lipopolysaccharide-stimulated endothelial cells induced the rapid shedding of neutrophil lectin adhesion molecule–1, the up-regulation of leukocyte β_2 integrins, and the attachment and transmigration of the neutrophils. Thus, endogenous endothelial IL-8 regulates transvenular traffic during acute inflammatory responses.

IN VIVO, INTERLEUKIN-1β (IL-1β), TUMOR necrosis factor α (TNFα), or lipopolysaccharide (LPS) trigger the endothelial cells that line the postcapillary venule to express adhesion molecules that can bind to circulating neutrophils (*1*). This initial adhesive interaction is regulated by a member of the lectin-cellular adhesion molecule family (lectin adhesion molecule–1; LECAM-1) that is displayed on the surface of unstimulated neutrophils (*1, 2*). Binding of the neutrophils to the inflamed endothelium then initiates an orchestrated series of events in which the neutrophils shed LECAM-1 and engage a second set of adhesion-promoting glycoproteins, the leukocyte β_2 integrins (*1, 2*). The adherent neutrophils then penetrate the vessel wall by moving between interendothelial-cell junctions and proceeding through the underlying basement membrane into the interstitium (*1–3*).

The initial interaction between LECAM-1 on the circulating neutrophil and the surface of stimulated endothelial cells is presumed to be a passive event (*1, 2*). However, the source and identity of the factors responsible for the initiation of the subsequent shedding of LECAM-1, the up-regulation of the β_2 integrins, and neutrophil diapedesis is unclear. We now show, in a model of the postcapillary venular wall (*3*), that IL-1β–, TNFα-, or LPS-stimulated endothelial cells actively promote neutrophil transvenular migration by the synthesis and release of a 77–amino acid variant of interleukin-8 ($IL\text{-}8_{77}$) (*4*). Although endothelial $IL\text{-}8_{77}$ is thought to be a specific inhibitor of neutrophil-endothelial cell interactions (*5*), we show that this molecule is a primary promoter of neutrophil diapedesis by virtue of its ability to both regulate LECAM-1 and β_2 integrin expression and to form a transendothelial cell chemotactic gradient.

To duplicate the conditions under which endothelial cells promote transvenular neutrophil migration in vivo, we added IL-1β, TNFα, or LPS to the basilar compartment of the blood vessel wall construct, and after a 4-hour incubation, 1×10^6 neutrophils were added in fresh media to the apical compartment, and invasion was monitored. Under these conditions, 79 ± 7%, 81 ± 6%, and 85 ± 8% (± SEM, $n = 4$) of the added neutrophils were associated with IL-1β–, TNFα-, and LPS-stimulated vessel wall constructs, whereas only 9 ± 3% were associated with untreated, control cultures (*6*). After 90 min, almost all of the vessel wall–associated neutrophils had successfully penetrated the endothelial layer and invaded the under-

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