

# Recurrent subarachnoid hemorrhage associated with a new transthyretin variant (Gly53Glu)

**Article abstract**—CNS involvement is rare in systemic amyloidoses due to transthyretin (TTR) mutation and manifests as a combination of dementia, seizures, and myelopathy. The authors report two French siblings who experienced recurrent subarachnoid hemorrhages as the main clinical feature. Brain specimens showed that the leptomeningeal vessels walls were thickened by amyloid deposits, and sequencing of the TTR exons showed a heterozygous single base-pair transition from G to A (codon 53), resulting in a glycine for glutamic acid substitution (G53E).

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Mutations in the gene of the transthyretin (TTR) result in autosomal dominant systemic amyloidoses. The most common phenotype, identified as familial amyloidotic polyneuropathy, mainly affects the peripheral nerve. Other organs such as kidney, heart, eyes, or spleen may be involved, but CNS is seldom affected.<sup>1,2</sup> Infiltration of TTR-amyloid in leptomeningeal vessels results in a combination of ataxia, spasticity, dementia, seizures, and myelopathy,<sup>3–5</sup> and recently, subarachnoid hemorrhage has been reported.<sup>6,7</sup> We describe two siblings who presented with recurrent episodes of subarachnoid hemorrhage and were affected by leptomeningeal amyloidosis. Analysis of the TTR gene indicated that a novel mutation at amino acid 53 of the molecule was responsible for this familial amyloidosis.

**Case reports.** *Patient 1.* A 43-year-old woman was admitted for recurrent, pseudomigrainous headaches of 6 years' duration, gait disorder, and a slowing of mentation. At age 41, she experienced severe headache of sudden onset, and a lumbar puncture indicated subarachnoid hemorrhage. Four-vessel angiography was normal, as was brain MRI at that time. Angiography was followed by transient aphasia and right incomplete hemiparesis, which lasted 12 hours.

On examination, the patient was orientated with a mild global impairment affecting attention and calculation. Her gait was ataxic, and reflexes were brisk. CT head scan disclosed calcifications along the left sylvian fissure (figure 1A). CSF was xanthochromic, and protein content was 3.1 g/L. MRI showed diffuse leptomeningeal enhancement (see figure 1, B and C), but cerebral parenchyma appeared normal. Echocardiography showed a marked hypertrophy of ventricles and septum suggestive of cardiac amyloidosis. A rectal biopsy evidenced amyloid deposits stained with anti-TTR antibodies. Vitreous examination and peripheral nerve conduction studies were normal.

During the following months, the patient's condition rapidly worsened, and she developed dementia, with severe headaches, spasticity, and urinary frequency. Despite a progressive deterioration, the clinical picture remained fluctuating even from day to day. Multiple episodes of impaired consciousness and drowsiness, lasting from 1 to 48 hours, were noted, some with visual hallucinations or focal transient deficits (aphasia and alternating hemiplegia). Cerebral MRI remained unchanged. Marked weight loss was observed, and the patient died at age 44. Autopsy was not performed.

*Patient 2.* A younger brother of Patient 1 had been admitted at age 42 for a spontaneous subarachnoid hemorrhage. He recovered without sequelae, and four-vessel angiogram was normal.

Neurologic examination at age 43 was normal, as were brain CT scan and contrast-enhanced MRI. Nerve conduction studies were normal, and vitreous opacities were absent. CSF protein content was 2.4 g/L. Echocardiography disclosed hypertrophic cardiomyopathy, and a rectal biopsy specimen showed anti-TTR-positive amyloid deposits. At age 44, he was readmitted for a coma, and CT head scan indicated subarachnoid hemorrhage (see figure 1D). He died 5 days later of recurrent bleeding. Analysis of post-mortem brain specimens showed that most of the leptomeningeal vessels walls were thickened by eosinophilic amyloid deposits (figure 2A). These lesions were also observed in a few superficial cortical vessels. Amyloid deposits were scattered in the leptomeninges and the subpial brain parenchyma. They were strongly immunopositive with anti-TTR antibodies (see figure 2B). Neither ischemic lesions nor amyloid angiopathy were observed in deep brain parenchyma.

*Patient 3.* An elder sister of Patients 2 and 3, aged 46, had chronic headaches for 10 years and experienced right arm weakness and dysphasia that lasted 15 minutes. Neurologic examination was normal, but MRI showed a focal meningeal enhancement in the left sylvian fissure.

The father of these three siblings had died at age 46 of a chronic neurologic illness beginning at age 41 with migraine-like headaches, walking disturbances, and dementia.

Genomic DNAs from the three affected siblings were extracted, and exons 2, 3, and 4 of the TTR gene were amplified by the PCR by using previously published primers and conditions.<sup>8</sup> Each PCR product was directly sequenced on both strands by using a dye terminator cycle sequencing kit (Perkin Elmer, Foster City, CA), and the sequencing products were resolved on an automatic fluo-

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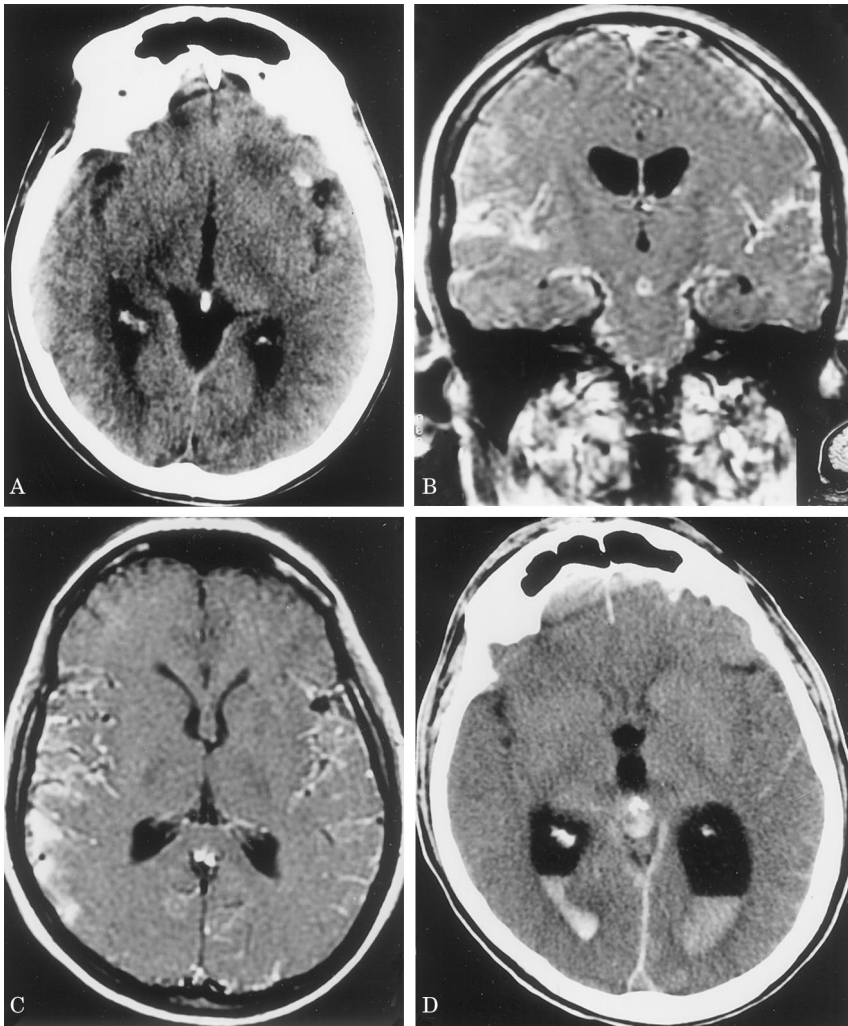


Figure 1. (A) Left perisylvian calcifications on axial nonenhanced CT scan of the head (Patient 1). (B and C) Coronal (B) and axial (C) T1-weighted images showing strong gadolinium enhancement of the leptomeninges (Patient 1). (D) Subarachnoid and intraventricular hemorrhage on axial nonenhanced CT scan of the head (Patient 2).

rescent DNA sequencer ABI Prism 377 (Applied Biosystems, Weiterstadt, Germany).

Sequence analysis of the TTR gene performed from the DNA of Patient 1 showed in exon 3 a heterozygous single base-pair transition from G to A, which predicts the replacement of glycine (G), a nonpolar residue, by glutamic acid (E), a polar residue, at codon 53 of the TTR molecule (G53E) (figure 3). Because this nucleotide substitution fortuitously creates a novel restriction site for the *MboI* en-

zyme in exon 3, *MboI* restriction analysis was used for rapid PCR-based testing of Patients 2 and 3, and both were shown to have the G53E mutation. Then the same technique was used for rapid PCR-based testing from 65 normal subjects to ensure that this sequence change was not a polymorphism. None were found to be positive for this mutation as well as 150 probands from unrelated families diagnosed with a TTR amyloidosis and for whom we previously identified the mutation. Therefore, the G53E

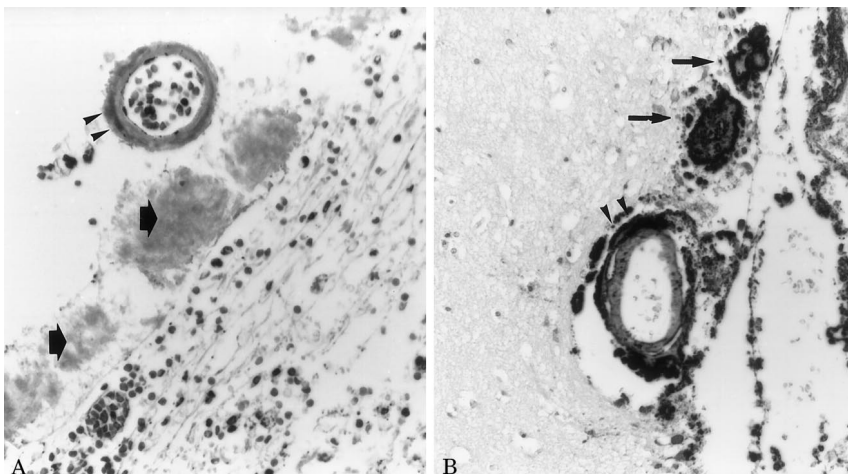
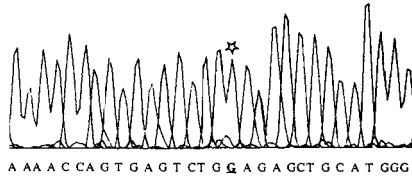


Figure 2. (A) Several amyloid deposits are seen in the leptomeninges (arrows) as well as in the external part of the vessel wall (arrowheads) (hematoxylin-eosin, original magnification  $\times 290$ , Patient 2). (B) Two large extravascular amyloid deposits (arrows) and a perivascular one (arrowheads) are strongly immunopositive with anti-TTR antibodies (immunoperoxidase technique, original magnification  $\times 180$ , Patient 2).

wild-type:

TCT GGA GAG



mutant:

52 53 54  
Ser Gly Glu  
allele 1: TCT GGA GAG  
allele 2: TCT GAA GAG  
Ser Glu Glu



Figure 3. Identification of the G53E variant of the TTR by direct sequencing of PCR products of exon 3 from Patient 1. Sequence chromatograms are shown on the right with the relevant wild-type and mutant partial sequences of TTR noted to the left. Asterisks point to the G-to-A transition.

mutation has been identified only in these three affected siblings, suggesting that it is most probably the causative mutation responsible for the leptomeningeal amyloidosis, although DNAs from healthy family members were not available.

**Discussion.** We report three patients with a meningocerebrovascular amyloidosis, shown, in two cases, by recurrent subarachnoid hemorrhage. Molecular investigations showed that this phenotype is associated with a novel mutation of the TTR gene (G53E).

Prominent CNS impairment is rare in systemic TTR-related amyloidoses and has been attributed to leptomeningeal infiltration. It remains difficult to predict whether a TTR variant could affect the CNS, because neither the type of the mutation nor the position of the amino acid substitution in the TTR gene seem to be specific features. Six different TTR variants have been associated with leptomeningeal amyloidosis.<sup>3,4,6,7,9,10</sup> Neurologic picture is heterogeneous, and a combination of ataxia, spasticity, and dementia is reported. Some of these cases are classified as oculoleptomeningeal amyloidosis on the basis of associated vitreous deposits.<sup>3,4</sup>

Although subarachnoid hemorrhage is not mentioned as a prominent feature, we believe that it is a frequent, often overlooked, clinical trait. An English patient experienced meningeal bleeding,<sup>6</sup> as did two Italian sisters with TTR-related (Ala36Pro) amyloidosis.<sup>7</sup> Four other patients were reported to suffer from migrainelike headaches with vomiting, which could correspond to minor meningeal bleedings.<sup>4,5</sup> Subarachnoid hemorrhage may be the first manifestation of the disease, occurring in the teenage years or in the third to fourth decade, and sometimes remains the only symptom (as in Patient 2).

Episodes of focal CNS dysfunction lasting from

hours to days seem to be a characteristic feature of this disease. In most cases, confusion and drowsiness occur, with aphasia, transient hemiplegia, and sometimes hallucinations.<sup>3,4,6</sup> Their mechanism remains unknown. As the disease progresses, the clinical picture becomes a combination of dementia, cerebellar and pyramidal signs, tremor, deafness, and incontinence. These symptoms may be attributed to siderosis of the brain<sup>7</sup> or compression of the spinal cord by thickened leptomeninges.<sup>10</sup> Although the prognosis is usually determined in TTR amyloidoses by the involvement of heart and kidney, CNS impairment and, specifically, recurrent subarachnoid hemorrhage were responsible for the death of our patients.

Neuroradiologic investigations may evoke the diagnosis. CT scan may detect nonspecific calcifications along the sylvian fissure,<sup>5</sup> but the diffuse meningeal enhancement on brain MRI is strongly suggestive of leptomeningeal amyloidosis.<sup>5,6,10</sup> However, it may be absent despite extensive pathologic evidence of leptomeningeal lesions, as for Patient 2. Diagnostic procedures may be harmful, likely because of the vessel weakness caused by amyloid deposits. Patient 1 had a transient deficit after angiography, and a fourth ventricle hemorrhage after lumbar puncture has been previously reported.<sup>7</sup>

Leptomeningeal amyloidosis caused by TTR gene mutation should be added to the list of causes of subarachnoid hemorrhage with normal angiograms. In recurrent forms, or when a familial trait is suspected, a search for systemic amyloid deposits (rectal biopsy, echocardiography) and molecular analysis of the TTR gene should be performed, even in the absence of peripheral neuropathy or vitreous deposits.

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