Transthyretin Leu12Pro is associated with systemic, neuropathic and leptomeningeal amyloidosis

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Summary
We report a middle-aged woman with a novel transthyretin (TTR) variant, Leu12Pro. She had extensive amyloid deposition in the leptomeninges and liver as well as the involvement of the heart and peripheral nervous system which characterizes familial amyloid polyneuropathy caused by variant TTR. Clinical features attributed to her leptomeningeal amyloid included radiculopathy, central hypoventilation, recurrent subarachnoid haemorrhage, depression, seizures and periods of decreased consciousness. MRI showed a marked enhancement throughout her meninges and ependyma, and TTR amyloid deposition was confirmed by meningeal biopsy. The simultaneous presence of extensive visceral amyloid and clinically significant deposits affecting both the peripheral and central nervous system extends the spectrum of amyloid-related disease associated with TTR mutations. The unusual association of severe peripheral neuropathy with symptoms of leptomeningeal amyloid indicates that leptomeningeal amyloidosis should be considered part of the syndrome of TTR-related familial amyloid polyneuropathy.

Keywords: familial amyloid polyneuropathy; oculoleptomeningeal amyloidosis; transthyretin

Abbreviation: TTR = transthyretin

Introduction
Familial amyloid polyneuropathy is the most common form of hereditary systemic amyloidosis. It is an autosomal dominant condition, usually caused by mutation in the gene for plasma transthyretin (TTR), although a few kindreds are known with a similar phenotype caused by apolipoprotein A1 gene mutations. Typical features of TTR-related familial amyloid polyneuropathy include severe somatic and autonomic peripheral neuropathy and variable but usually modest amyloid involvement of the spleen, kidneys, heart, eyes, adrenals and thyroid gland. Hereditary oculoleptomeningeal amyloidosis is a rare syndrome in which there are opacities of the vitreous humour and CNS symptoms associated with leptomeningeal amyloidosis. Oculoleptomeningeal amyloidosis is less well characterized than familial amyloid polyneuropathy, but may also be associated with TTR mutations (Goren et al., 1980; Uitti et al., 1988; Petersen et al., 1995; Herrick et al., 1996; Vidal et al., 1996). Here we report an English patient with a novel TTR mutation who had classical features of familial amyloid polyneuropathy in addition to those of oculoleptomeningeal amyloidosis. She also had severe systemic amyloidosis, including amyloid deposition in the liver. Although >95% of circulating TTR is produced by the liver, significant parenchymal amyloid deposition has not been reported as affecting the liver in patients with amyloidogenic TTR mutations. Her phenotype supports the idea that there is a continuum of pathology associated with amyloidogenic TTR mutations, and her major system involvement further extends the spectrum of amyloid disease that may occur in this setting.

Case report
Our index case was 38 years old when she first began to notice easy bruising. Five years later, in 1985, she began to get persistent headaches, and 6 months after this presented...
with severe headache of sudden onset. CT and lumbar puncture confirmed subarachnoid blood, but her angiogram showed no definite bleeding point, and she was managed conservatively. Two months later, she had another subarachnoid bleed, but her angiogram was unchanged. She remained well after discharge until 1990, when she started to notice hearing loss in both ears, increasingly severe headaches, unsteadiness, urinary frequency, incomplete bladder emptying and poor urinary stream. A CT scan of her brain showed hydrocephalus, and insertion of a right lateral ventriculoperitoneal shunt was complicated by a small subdural haematoma and slow recovery from anaesthesia. After the shunt, her unsteadiness and urinary symptoms partially improved, but she began to notice a dry mouth, dry eyes, constipation and orthopnoea.

In 1992, she became increasingly nauseated and unsteady when walking, and by the middle of 1993 she noticed weakness and numbness in her feet. A CT scan showed a contracted lateral ventricle on the shunted right side, but the rest of her ventricular system was dilated. She received a left lateral ventricular shunt, again complicated by some bleeding. Her ventricular CSF had a raised protein level of 1.1 g/l; CSF protein from her earlier lumbar punctures had been normal. After the operation, she developed urinary retention and had to be catheterized for a few days.

There was little improvement of her symptoms after the second shunt. At the end of 1993, she started to get floaters in both eyes and, in May 1994, at the age of 51, she was admitted to hospital after several weeks of headaches and intermittent confusion. She had patchy sensory loss in the feet, marked ataxia of gait, and was in painless urinary retention. A CT showed no hydrocephalus, and chest X-ray showed cardiomegaly with pleural effusions. Her ECG had anterior Q waves with lateral T wave inversion, and an echocardiogram showed a thickened septum and posterior wall, both typical of cardiac amyloid (Staunton, 1991). Several lumbar punctures over the course of her admission found CSF protein values between 4 and 19 g/l, whereas ventricular CSF drawn from her shunt reservoirs had protein values of 1.1–1.4 g/l, suggesting an element of spinal CSF block. An enhanced MRI scan showed striking enhancement of both cerebral and spinal meninges (see Figs 1 and 2).

On examination, she had markedly dry eyes and mouth, and several areas of bruising. She had postural hypotension, with a supine blood pressure of 90/50 dropping to 50/30 on standing. There were signs of moderate biventricular cardiac failure. She was alert and orientated; both her fundi were partially obscured by vitreous debris and her pupils did not react to light, but responded slowly to accommodation. She had moderate bilateral sensorineural hearing loss and slow tongue movements. Her tone was normal but there was distal wasting and weakness. Co-ordination was slightly impaired in her arms, and she had a markedly ataxic gait. Her reflexes were sluggish throughout, and ankle jerks were only present with reinforcement; both plantar responses were flexor. There was a patchy loss of light touch and pinprick in a glove and stocking distribution, with a moderate reduction of temperature sensation. Joint position sense was reduced in her hands, and in her feet up to her knees. Formal psychometry revealed an average IQ, which represented a fall from her estimated pre-morbid level.

Nerve conduction studies demonstrated reduced amplitude sensory action potentials in the hands, and absent sensory action potentials in the sural nerves. Conduction velocities were normal and there was evidence of distal denervation on EMG. Paraspinal and intercostal EMG showed some fibrillation, increased insertional activity and complex repetitive discharges in all muscles sampled. These paraspinal denervation changes were felt to be significantly greater than would have been expected from the degree of distal denervation, and were thus suggestive of radiculopathy. The overall picture was, therefore, of an axonal sensorimotor neuropathy combined with a diffuse radiculopathy. Autonomic function tests confirmed postural hypotension, and a blocked heart rate response to cold, to mental arithmetic and to the Valsalva manoeuvre.

In June of 1994, she had a right-sided focal seizure with altered consciousness, and some left temporal EEG changes. Later that month, she had a posterior fossa meningeal biopsy.
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Fig. 2 Gadolinium-enhanced T1-weighted MRI of the brain. There was extensive enhancement from the surface of the brain, and of the fourth and lateral ventricles.

After the operation, she remained intermittently confused and drowsy for 9 days, and blood gases showed her to be in variable type 2 respiratory failure. Two days after her operation, she had episodes of alternating apnoea and hyperventilation which gradually resolved over the following week. She subsequently continued to suffer from severe night time hypoxia, and continuous monitoring of her oxygen saturation showed prolonged cycles of hypoxia with saturations as low as 40%, suggesting that there was a significant central component to her reduced ventilation.

Histology of the meningeal specimen showed extensive leptomeningeal amyloid (see Results). She later went on to have a vitrectomy which also showed amyloid on staining with Congo Red. Immunohistochemical staining of the meningeal amyloid deposits demonstrated TTR, and direct DNA sequencing revealed a mutation in the TTR gene.

In January 1995, she became very depressed. Later that month, she became increasingly drowsy and hypoxic. The reason for the confusion was not clear; blood gas results were adequate on continuous inhaled oxygen, a repeat CT scan showed no hydrocephalus and the biochemistry was unremarkable. Mineralocorticoid treatment had been started, and supine blood pressure was adequate at 130/100, although she still had severe symptomatic postural hypotension. She continued to be troubled by dyspepsia, constipation and nausea. The prognosis was clearly very poor, and after full discussion the family declined to consider liver transplantation, and decided that she should have no further active treatment or investigation. However, she did not deteriorate, and in April 1995 she began to become less confused. This seemed to fluctuate from day to day. When she was coherent, she continued to be severely depressed. In April, she had a second partial seizure. By the end of June, her mental state had improved and she was consistently alert and oriented. In July 1995, she was discharged, and died at home 5 months later at the age of 53 years. There was no post-mortem.

Family history revealed that the patient’s mother had committed suicide at the age of 62 after 2 years of depression and physical illness, including minor urinary symptoms, constipation and falls. She had been admitted to hospital at the age of 61 with severe abdominal pain, and while in hospital had two episodes of unexplained confusion. She complained of flashing lights and spots in front of her eyes, although neurological and ophthalmological examinations were recorded as normal. A year later she was admitted to the local psychiatric hospital with severe depression, and committed suicide a few months later. The post-mortem report has been lost, but tissue blocks preserved from her heart, lung and kidney were obtained for study (see below). There was no other family history of psychiatric or neurological disease. The family was unwilling for us to examine or investigate other family members, and declined genetic counselling.

Methods

Histology
Amyloid was identified by green birefringence on sections stained with Congo Red viewed in polarized light (Puchtler et al., 1962). TTR immunoreactivity was identified as previously described (Booth et al., 1995). Sections were stained similarly using antisera to immunoglobulin light chains and serum amyloid A protein.

Serum amyloid P component scintigraphy
Whole body scintigraphy following administration of 123I-labelled serum amyloid P component was performed as
previously described (Hawkins et al., 1988, 1990). Approximately 150 MBq of $^{123}$I-labelled serum amyloid P component was injected, and anterior and posterior whole body images were obtained 24 h after administration.

**Sequencing of the TTR gene**

DNA was extracted from whole blood and the four exons of the entire TTR gene were amplified by PCR (polymerase chain reaction) using *Taq* polymerase as previously described (Booth et al., 1995). Aliquots of 100 µl of the PCR products were purified by size fractionation on an agarose gel and the DNA bands subsequently were used for the sequencing reaction using the appropriate primer. A reaction mixture containing 2 µl of primer, 2 µl of sequencing buffer and 6 µl of template was boiled for 2 min, frozen in a dry ice–methanol bath and 5 µl of Mastermix (Amersham Life Sciences, St Albans, UK) was added. Just after thawing, 3 µl of the mixture was added to 2.5 µl of each of the four dideoxynucleotides before incubating the termination reaction at 37°C for 2 min, and finally adding 4 µl of stop solution. The same primers were used for PCR and sequencing, except for exon 4 where a different primer gave a better sequence analysis.

**Results**

**Histology**

A posterior fossa meningeal biopsy provided membranous fragments of grey tissue measuring 0.7 cm across. Most of the specimen was composed of rather paucicellular tissue with dense parallel collagen bundles suggestive of an origin in dura mater, and separate fragments of thickened leptomeninges were also present. Moderate amounts of acellular, eosinophilic material were deposited around dural blood vessels and much more abundantly in the leptomeninges (Fig. 3), and this was confirmed as amyloid by Congo Red staining.

There was specific staining of the deposits with the anti-TTR serum which was abolished by prior absorption with TTR. There was no specific staining with the antisera to immunoglobulin light chains or serum amyloid A protein. No amyloid was detected in sections of heart, lung and kidney from the mother’s post-mortem material.

**Serum amyloid P component scintigraphy**

Serum amyloid P component scintigraphy showed quite intense abnormal uptake of tracer in the liver, spleen and kidneys, indicating the presence of substantial amyloid deposits in these sites. Liver amyloid has not been demonstrated by this method in any of the >60 patients with other amyloidogenic TTR mutations who have been studied in the Immunological Medicine Unit at Hammersmith Hospital.

**Gene sequencing**

Amplification and direct sequencing of all four exons of the TTR gene showed that our patient was heterozygous for a single base change in one allele of exon 2, altering the codon for residue 12 of the native protein from Leu (CTG) to Pro (CCG). The remainder of the sequence was normal.

**Discussion**

More than 60 variant forms of TTR have now been identified, >80% of which are associated with hereditary amyloidosis (Benson and Uemichi, 1996). Although some such mutations present predominantly with cardiac amyloidosis, familial amyloid polyneuropathy is by far the most common syndrome associated with variant TTR.

The clinical picture of familial amyloid polyneuropathy was first described by De Bruyn and Stern (1929), although they misdiagnosed their case as Déjerine–Sottas disease (De Navasquez and Treble, 1938). Andrade (1952) accelerated the study of the disease with his classic description of cases in the Oporto region of Portugal. It subsequently was shown that Andrade’s patients had a variant TTR (Costa et al., 1978) with a Met30Val substitution in the mature protein (Dwulet and Benson, 1983). Since then, many different mutations of TTR have been associated with familial amyloid polyneuropathy (Benson and Uemichi, 1996).

Staunton (1991) and Reilly (Reilly and Staunton, 1996) recently have reviewed the clinical aspects of TTR-related familial amyloid polyneuropathy. The age of onset varies between families, from the third decade (Andrade, 1952; Silva Horta et al., 1964) to the sixth (Staunton et al., 1987). By definition, all kindreds with familial amyloid polyneuropathy suffer from peripheral neuropathy, which is an axonal sensorimotor polyneuropathy (Thomas and King, 1974; Staunton et al., 1987), and usually begins in the feet. Some families develop early carpal tunnel syndrome (Rukavina et al., 1956). Autonomic dysfunction is common and presents with impotence, gastrointestinal symptoms or postural hypotension. There is often amyloid cardiomyopathy, which may cause heart failure and rhythm disturbances. Some families suffer from vitreous opacities (Gorevic and Rodrigues, 1994). Amyloid nephropathy may be prominent.

There are two other proteins that are known to be associated with familial amyloid polyneuropathy. Variant gelsolin causes lattice corneal dystrophy and cranial neuropathy, followed by peripheral neuropathy (Meretoja, 1969; Maury et al., 1990). Mutations in apolipoprotein A1 are sometimes associated with a polyneuropathy similar to that of TTR-related familial amyloid polyneuropathy, although most affected patients have very extensive systemic amyloidosis involving many organ systems (Van Allen et al., 1969; Nichols et al., 1990).

Until recently, it was thought that CNS symptoms were not a major feature of TTR-related amyloidosis. However, it recently has become clear that the syndrome of
oculooleptomeningeal amyloidosis is associated with TTR mutations. Goren et al. (1980) were the first to use the term oculooleptomeningeal amyloidosis to describe a syndrome of familial systemic amyloid that caused symptoms by involving the vitreous humour and the leptomeninges. Apart from Goren’s report, there are now five other families in the literature which fit this description (Hamburg, 1971; Herrick et al., 1996; Okayama et al., 1978; Uitti et al., 1988; Vidal et al., 1996).

Oculooleptomeningeal amyloidosis has presented from the third (Uitti et al., 1988) to the seventh decade (Herrick et al., 1996). The clinical picture in these patients varies considerably from patient to patient, even within a kindred, but common features include vitreous opacities, headaches, progressive dementia, convulsions, ataxia, spasticity and, characteristically, episodes of fluctuating consciousness often associated with focal neurological signs. Peripheral somatic and autonomic neuropathy have been reported in some of these patients, but were typically mild. Post-mortem findings were of systemic amyloidosis, with variable degrees of peripheral nerve involvement. The characteristic finding was of extensive amyloid thickening of the leptomeninges and subarachnoid vessels. Amyloid in the blood vessels disappeared as the vessels penetrated the parenchyma, and there was diffuse neuronal loss in the CNS parenchyma which was most severe in the superficial layers. Most cases had a moderate degree of hydrocephalus, and some had superficial infarcts of the cerebral and cerebellar cortex.

In three families with oculooleptomeningeal amyloidosis, a TTR mutation has been identified. Vidal et al. (1996) found an Asp18Gly mutation in their kindred, Herrick et al. (1996) reported a Val30Met substitution, and the patients of Goren et al. (1980) had a Val30Gly substitution (Petersen et al., 1995). In two other reports of oculooleptomeningeal amyloidosis, there was some evidence of an abnormal TTR (Okayama et al., 1978; see Kitamoto et al., 1986; Uitti et al., 1988). There are no data on the nature of the amyloid protein from the report of Hamburg (1971).

Our patient had the characteristic clinical features of TTR-related familial amyloid polyneuropathy. She had an ascending axonal sensorimotor polyneuropathy and severe autonomic dysfunction, with urinary symptoms, constipation, Sjögren’s (sicca) syndrome, postural hypotension and tonic pupils. She had amyloid vitreous opacities, mild renal impairment and cardiac failure, with typical ECG and echocardiogram findings for amyloid cardiomyopathy (Staunton, 1991). She also had many clinical features that are not part of the TTR familial amyloid polyneuropathy syndrome, but are suggestive of oculooleptomeningeal amyloidosis. Thus she had recurrent subarachnoid haemorrhage, a high CSF protein, hydrocephalus, probable radiculopathy, reduced respiratory drive, episodic reduction in conscious level, fluctuating confusion and severe depression. A meningeal biopsy confirmed that she had extensive amyloid deposits in her meningeal vessels and within the dura, and MRI scans showed very widespread meningeal enhancement. The MRI and biopsy findings suggest that she had similar meningeal pathology to that in patients with oculooleptomeningeal amyloidosis. However, some features of our case are unusual for both syndromes, and merit further comment.

Our patient is the first reported with recurrent subarachnoid haemorrhage attributed to leptomeningeal amyloid. Although this feature is almost unique to our patient, it is well known that blood vessels infiltrated by amyloid are likely to bleed in amyloid angiopathy associated with both Cystatin C4 and Aβ amyloidosis. There is also some evidence for this in previous reports of oculooleptomeningeal amyloidosis and
familial amyloid polyneuropathy. Among Uitti’s cases of oculoleptomeningeal amyloidosis (Uitti et al., 1988), one patient had a large frontal intracerebral bleed, and another had an old occipital haematoma at autopsy. Koeppen et al. (1985, 1990) reported that a patient with TTR-related familial amyloid polyneuropathy had died from a subarachnoid haemorrhage at the age of 43. A Japanese patient with familial amyloid polyneuropathy died of a subarachnoid haemorrhage (Ikeda et al., 1987), but this was ascribed to a thoracic arteriovenous malformation found at post-mortem. Arpa Gutierrez et al. (1993) described a patient with TTR-related familial amyloid polyneuropathy who had had a pontine haemorrhage in her fifth decade. At post-mortem, she had extensive leptomeningeal amyloid, and amyloid in the vessels around the haemorrhage.

Episodes of impaired consciousness were a prominent symptom in our patient, as in previous cases of oculoleptomeningeal amyloidosis. No convincing explanation has yet been offered for this phenomenon. In past reports, these episodes may last from hours to many days, and they are often associated with focal signs suggesting cortex or brainstem involvement (Krücke, 1950; Hamburg, 1971; Okayama et al., 1978; Goren et al., 1980; Uitti et al., 1988). Our patient was admitted with a complaint of intermittent confusion, and had two significant periods of drowsiness and confusion while in hospital. Goren et al. (1980) suggested that such episodes in their patients might have been due to intermittent hydrocephalus. However, observations in our patient do not support this mechanism, because she suffered similar episodes when she had two functioning ventriculo-peritoneal shunts. It is also unusual for hydrocephalus to cause the focal deficits that occur in oculoleptomeningeal amyloidosis such as alternating hemiparesis or aphasia (Okayama et al., 1978; Goren et al., 1980; Uitti et al., 1988). It is possible that these episodes represent transient episodes of ischaemia of the cortex and brainstem. In our patient, we postulate that the respiratory failure and reduced level of consciousness were both due to episodes of brainstem ischaemia. Post-mortem findings in patients with oculoleptomeningeal amyloidosis often show narrowing and occlusion of cerebral blood vessels, with some recanalization (Goren et al., 1980). It is possible that this vessel narrowing coupled with reduced autoregulation by the amyloid-laden vasculature may lead to prolonged ischaemia without infarction.

Our patient may well have had radiculopathy as well as moderate axonal neuropathy, and there is some evidence for radiculopathy in other patients with oculoleptomeningeal amyloidosis. Uitti et al. (1988) reported paraspinal muscle atrophy in their index case, and Goren et al. (1980) reported L5 radiculopathy in one of their patients. Radiculopathy can be difficult to detect both clinically and by electrophysiology when there is also a peripheral neuropathy. It has been noted previously that in familial amyloid polyneuropathy there is little relationship between the severity of neuropathy and the amount of amyloid in peripheral nerves (Staunton, 1991). Some post-mortem studies in familial amyloid polyneuropathy have shown extensive disruption of the dorsal root ganglia and motor roots (De Navasquez and Treble, 1938; Juliao et al., 1974; Takahashi et al., 1991), and this may be a major factor in the neuropathy of familial amyloid polyneuropathy (Thomas and King, 1974; Staunton, 1991).

Findings from the imaging studies of our patient were also striking. The enhanced MRI scan of her brain and spinal cord was very unusual, with profuse and widespread enhancement of the meninges, consistent with the widespread amyloid deposits in meninges and blood vessels described in post-mortem studies of oculoleptomeningeal amyloidosis. Herrick et al. (1996) recently have reported similar MRI findings in their patient with symptoms of oculoleptomeningeal amyloidosis. It therefore seems likely that this picture is characteristic of leptomeningeal amyloidosis. The appearance of the radiolabelled serum amyloid P component scan was also unusual; not only were the amyloid deposits more extensive than is usually seen, but hepatic amyloid deposits have not been identified previously by these means in patients with TTR mutations (Hawkins, 1994; Hawkins et al., 1996) or found within the liver in histological studies. Although the liver is the most important site of TTR synthesis, there are presumably factors within the microenvironment of the hepatic parenchyma that do not favour amyloid fibril formation. The extensive nature of our patient’s systemic amyloid deposits, including the liver involvement, may simply reflect the extremely prolonged course of her disease, which may have contributed to the development of her CNS features.

Our patient differs from previous cases of oculoleptomeningeal amyloidosis in that she had prominent somatic and autonomic peripheral neuropathy. She therefore has a phenotype that is intermediate between typical TTR-related familial amyloid polyneuropathy and oculoleptomeningeal amyloidosis. This suggests that leptomeningeal amyloid may not be a distinct clinical entity, but part of a spectrum of TTR disease. There are three lines of evidence to support this conclusion. First, the post-mortem findings from patients with classical familial amyloid polyneuropathy often show severe leptomeningeal amyloid (Silva Horta et al., 1964; Juliao et al., 1974; Benson and Cohen, 1977; Takahashi et al., 1991; Ushiyama et al., 1991; Arpa Gutierrez et al., 1993; Benson, 1996). Secondly, one patient with symptoms of oculoleptomeningeal amyloidosis has a TTR mutation (Val30Met) which is commonly associated with classical TTR familial amyloid polyneuropathy (Herrick et al., 1996). Thirdly, there are a few other patients in the literature with classical familial amyloid polyneuropathy and symptoms of leptomeningeal disease. Götze and Krücke (1941; Krücke, 1950, case 1) described the case of a sculptor who suffered a progressive autonomic and somatic peripheral neuropathy. He had marked affective change and memory impairment, and was unable to keep track of events in the news, although he was living in the Germany of 1939. Later, Krücke (1950, case 2) reported another patient with an autonomic and somatic sensory peripheral neuropathy, cardiomyopathy,
headaches, seizures, and an episode of meningism and altered consciousness. Autopsy of both patients showed severe leptomeningeal amyloid with superficial cortical atrophy and infarcts. Kantarjian and DeJong (1953) described a family with somatic and autonomic peripheral neuropathy, vitreous opacities, depression and high CSF protein. A limited post-mortem on one of their cases found systemic amyloid and fibrosis of the spinal cord, although there is no comment on the brain or leptomeninges. There are other reports of patients with familial amyloid polyneuropathy and early strokes (Ikeda et al., 1987, case II-5 of family T; Arpa Gutierrez et al., 1993).

Lastly, it is notable that our patient has a new TTR mutation and an unusual phenotype. The relationship between the two is not clear. However, it is possible that specific mutations of TTR may be more likely to cause leptomeningeal amyloidosis. The substitution of proline at position 55 in the TTR molecule is associated not only with a very aggressive disease in vivo (Jacobson et al., 1992), but also a significant loss of TTR tetramer stability in vitro, promoting denaturation to a putative amyloidogenic intermediate and thus increasing amyloidogenicity (McCutchen et al., 1993, 1995). The apparent amyloid-promoting feature of this substitution may relate to the ability of proline to destabilize β strands (Wood et al., 1995), which are a prominent feature of wild-type TTR. This effect may also result from the creation of a proline–proline doublet at positions 11–12, as predicted by the DNA sequence for the variant reported here. Finally, it is of interest that Pro12 represents the third mutation in the TTR gene which causes changes in the A strand of the mature protein (Booth et al., 1996; Vidal et al., 1996), a region in which amino acid substitutions previously were considered unlikely to have a significant influence on the stability of the protein.

References

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