

Progressive myoclonus epilepsy associated with neuroserpin inclusion bodies (neuroserpinosis)

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ABSTRACT – Familial encephalopathy with neuroserpin inclusion bodies (FENIB) is a conformational proteinopathy characterised by neuronal inclusion bodies composed of the serine protease inhibitor (SERPIN), neuroserpin. Presenting clinically as a familial dementia-epilepsy syndrome, the molecular mechanism of the pathogenic abnormalities in neuroserpin has been characterised at atomic resolution. There is a remarkable genotype-phenotype correlation between the degree of molecular destabilisation of the several variants of the neuroserpin protein, their propensity to self-associate and the age of onset of the dementia-epilepsy complex. As with other serpinopathies there appears to be a mix of cell-autonomous toxicity, due to neuronal accumulation of neuroserpin, and non-cell autonomous toxicity, caused by loss of protease inhibition, in this case the dysregulated protease is likely to be tissue plasminogen activator (tPA). FENIB should be considered in cases of progressive myoclonic epilepsy and dementia particularly where there is family history of neuropsychiatric disease.

Key words: serpins, serpinopathy, protease inhibitor, conformational disease, neuroserpin, dementia, familial progressive myoclonic epilepsy, FENIB, progressive myoclonus epilepsies

Familial encephalopathy with neuroserpin inclusions bodies (FENIB) has been identified as a cause of pre-senile dementia with frontal symptoms as well as progressive myoclonic epilepsy (Minassian *et al.*, 2016). In affected individuals, mutated neuroserpin accumulates in neuronal inclusions (Collins bodies). FENIB is due to mutations in the *SERPIN1* gene located on chromosome 3q26. Serine protease

inhibitors (serpins) are a large superfamily of proteins that employ a conserved molecular mechanism for the inhibition of a wide range of proteases. Mutations may render serpins prone to polymerisation, *i.e.* ordered aggregation, in the endoplasmic reticulum of the synthetic Acell. In the case of neuroserpin (NS), a neuronal protein, this aggregation causes gain-of-function neuronal dysfunction

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that is thought to underpin the neurological manifestations. NS also inhibits tPA and, in this way, is thought to regulate the sensitivity of neurones to glutamatergic excitatory neurotransmission at the NMDA (N-methyl-D-aspartate) receptor. The epileptic component of FENIB may be due to dysfunction of the NS/tPA pathway.

Neuroserpin (NS) is a member of the serine protease inhibitor, or serpin, superfamily and has sequence and structural homologies to the archetype α_1 -antitrypsin. At the molecular level, serpins are composed of 9 α -helices, 3 β -sheets (A to C) and an exposed mobile reactive centre loop (RCL). The RCL typically contains 20 residues that act as a pseudo-substrate for the target protease (Elliott *et al.*, 1996; Ryu *et al.*, 1996). The main role of NS is to regulate the plasmin proteolytic pathway, in particular by inhibiting tissue-type plasminogen activator (tPA). In this regard, NS plays physiological roles in the development of the central nervous system (Seeds *et al.*, 1999), in learning and memory and also in such pathological events as stroke (Cole *et al.*, 2007) and epilepsy (Qian *et al.*, 1993).

Numerous mutations in human serpins have been linked to a wide range of diseases; examples include emphysema, angioedema, and dementia with progressive myoclonus epilepsy, resulting from mutations in α_1 -antitrypsin, C1-inhibitor, and neuroserpin, respectively. Direct toxicity is invariably a consequence of the intracellular accumulation of serpin aggregates that are termed polymers, and may result in death of the synthetic cell. In the case of neuroserpin, cytotoxicity is seen exclusively in neurons of the central nervous system. There may also be associated loss-of-function effects caused by the deregulated hyperactivity of the target proteases and this may underpin the development of epilepsy in patients carrying neuroserpin variants. To date, only a few families and exceptionally rare non-familial cases of progressive myoclonic epilepsy linked to the *SERPIN1* gene have been described. Age at onset is between 13 and 60 years and the disorder is severe with rapid loss of autonomy and premature death.

Pathophysiology of neuroserpin-related disease

Neuroserpin was first identified as an axonally-secreted glycoprotein in the conditioned medium of embryonic chick dorsal root ganglia cells (Stoeckli *et al.*, 1989; Osterwalder *et al.*, 1996). In mammals it is expressed in the central and peripheral nervous system, predominantly in neurones (Hastings *et al.*, 1997; Osterwalder *et al.*, 1998) where it can inhibit a number of trypsin-like enzymes, including thrombin and plasmin (Osterwalder *et al.*, 1998), however, subsequent

studies indicate that PA is the main physiological target. Accordingly, the highest levels of NS are found in parts of the nervous system that have the highest expression of tPA mRNA and protein (Krueger *et al.*, 1997).

While not all serpins interact with proteases, those such as neuroserpin, which have retained inhibitory activity, employ a highly conserved molecular mechanism. The sequence of events that results in inhibition begins with the serpin's RCL (labelled in red in *figure 1M*) entering the active site of the cognate protease, initially behaving as a substrate. The enzyme and serpin form a transient intermediate called the Michaelis complex (Ye *et al.*, 2001) which precedes the cleavage of the RCL at a specific position, termed the P1-P1' bond. This leads to the release of the P1 residue and the formation of an ester bond between the C-terminal portion of the serpin and the protease. At this stage, however, further hydrolysis, that would otherwise release cleaved serpin and active protease, is prevented by a profound conformational change in the serpin (Wright & Scarsdale, 1995). This transition from a "stressed to relaxed" form occurs as the protease-attached remainder of the RCL inserts as a α -strand in the main β -sheet A of the serpin core. This insertion is highly energetically favourable and violently flips the enzyme from the upper to the lower pole of the serpin (Huntington *et al.*, 2000), causing steric denaturation and inactivation of the protease. Permanent destruction of the protease is subsequently achieved by third-party proteolysis of those domains of the target protease that have been rendered unstructured (Huntington *et al.*, 2000). Experimentally, the enzyme-serpin complex is relatively stable with a dissociation rate constant that is an order of magnitude slower than the association rate (Belorgey *et al.*, 2002). However, dissociation of the serpin-enzyme complex does occur, in which case the released neuroserpin is in the inactive, cleaved conformation. In contrast, when tPA is liberated from the complex, it retains its proteolytic activity (Osterwalder *et al.*, 1998).

Clinical features and genotype-phenotype correlations

Mutations in the neuroserpin gene were first reported to cause an autosomal dominant form of pre-senile dementia (Belorgey *et al.*, 2002), described as familial encephalopathy with neuroserpin inclusion bodies (FENIB), characterized histologically by unique neuronal inclusion bodies and biochemically by polymers of the neuron-specific serpin, neuroserpin. These authors reported 2 unrelated Caucasian families living in the United States, carrying 2 different heterozygous mutations (S49P and S52R). In the larger family, affected individuals presented clinically around the

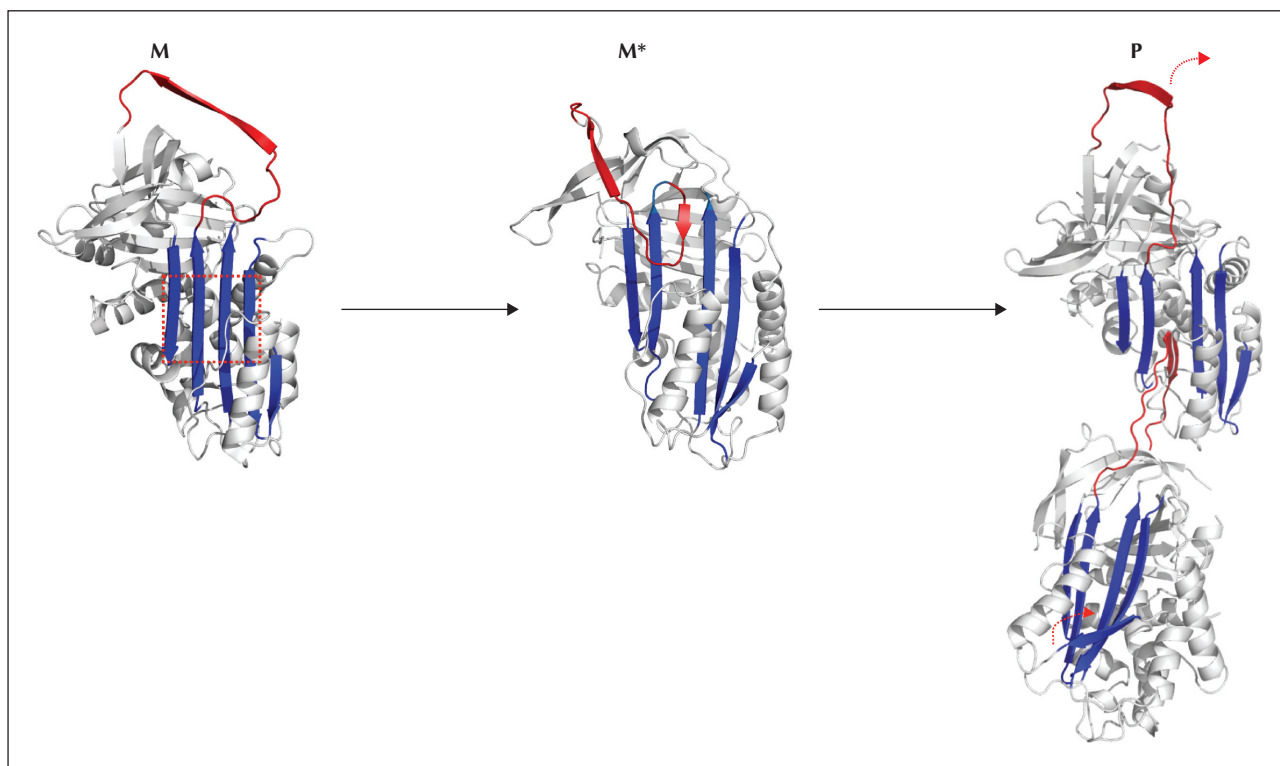


Figure 1. Molecular models of serpin conformers. M represents the active protease inhibitor with the reactive loop shown in red and β -sheet A in blue. Mutations that destabilize sheet A (typically in the so-called 'shutter region' signified by the red dashed box) allow the partial insertion of the reactive loop to form a new strand in sheet A (M^*). The consequent opening of the sheet allows the reactive loop from another serpin to insert forming a serpin dimer (P). The repeated addition of serpins by loop-sheet polymerisation in this way results in large aggregates.

fifth decade of life with cognitive decline, including deficits in attention and concentration, response regulation difficulties, and impaired visuospatial skills. Memory was also impaired, but to a lesser degree than is typically seen in individuals with Alzheimer's disease. After several years of disease progression, most affected individuals were unable to work and eventually required nursing-home care. The second, much smaller family showed an earlier clinical onset, during the second or third decade of life. Affected individuals presented with both epilepsy and progressive cognitive decline and their neuro-histology was dominated by eosinophilic, PAS-positive intraneuronal inclusions in the brain. Later on, a small family with 2 affected siblings featuring progressive myoclonus epilepsy and dementia was described (Belorgey *et al.*, 2002). The affected individuals developed generalized seizures in adulthood and progressed to status epilepticus over several years. In addition, they also developed slow speech, diplopia, nystagmus, dysarthria, and myoclonus in the extremities, with rapidly progressive dementia. Their deceased mother was, reportedly, similarly affected.

Davis *et al.* (1999a) reported additional patients with the disorder: a 23-year-old man with an 8-year history of

progressive myoclonic epilepsy, dementia, tremor, and dysarthria. The second patient was a 13-year-old girl with progressive myoclonus epilepsy with intractable seizures, myoclonus, and dementia. She died at the age of 19 during status epilepticus. Her father was said to be 'mentally deficient', and a paternal uncle had died from epilepsy at the age of 18.

Gourfinkel-An *et al.* (2007) subsequently described a small French family with progressive myoclonic epilepsy associated with a frontal syndrome starting from the age of 18 with severe myoclonus, generalized tonic-clonic seizures, and absences. The EEG of one of the patients showed diffuse slow waves, spikes, and spike-wave discharges superimposed on a slow background, with photic sensitivity at around 1 Hz. Cerebral MRI revealed cortico-subcortical atrophy. The patient's condition progressively worsened and swallowing difficulties were noticed at an early stage. Additionally, cerebellar symptoms and pyramidal signs were also present. Cognitive deterioration was severe (Mini-Mental Status Examination score: 12/30). Signs of frontal dysfunction were observed (emotional lability, distractibility, and poor performance on sequential motor tests) with sparing of long-term explicit memory. In another affected relative, who died at the age of 33,

Table 1. Various mutations in the neuroserpin gene cause onset of dementia and myoclonic epilepsy at a wide range of ages. The most aggregation-prone neuroserpin variants *in vitro* cause juvenile forms of FENIB while less destabilizing mutations are not penetrant until the seventh decade. Histologically, patients with early onset disease exhibit a higher burden of cortical neuroserpin inclusions (+ through to ++++).

Mutation	Onset (years)	Clinical findings	Inclusions
S49P	45-63	Dementia and terminal seizures	+
S52R	20-40	Myoclonus, Dementia	++
L47P	20-30	Progressive myoclonus epilepsy	+++
H338R	15	Progressive myoclonus epilepsy	+++
G392E	13	Progressive myoclonus epilepsy, chorea	++++
G392R	8	Epilepsy with slow wave sleep	++++

symptoms were similar and were first noticed when she was 18. Behavioural problems, depression, and frontal dysfunction were noticed. Epilepsy was drug-resistant and she experienced, during the course of the disorder, two episodes of status epilepticus. She became mute and bedridden and died of inhalation pneumonia at the age of 33. In these patients, a heterozygous S52R missense mutation at position 273 in exon 2 was detected, the same as in the two families from the United States.

A few non-familial cases have been also described. Coutelier *et al.* (2008) reported an 11-year-old girl who had had normal development until 8 years of age, when she developed a rapidly-progressive symptomatology, including aggressive behaviour, intellectual decline, psychic seizures, and subtle seizures with eyelid myoclonus. The EEG was suggestive of epilepsy with continuous spike-and-waves during slow-wave sleep. This patient was the first person identified with the G392R mutation in neuroserpin that resulted in severe juvenile phenotype and had accordingly appeared *de novo*. Hagen *et al.* (2011) more recently reported an additional sporadic patient with progressive myoclonus epilepsy and declining mental status starting in adulthood. Generalized seizures occurred early with myoclonus and progressive gait disturbances. Neuroimaging revealed mild atrophy and multiple periventricular white matter lesions, consistent with demyelination. The course was one of progressive decline with death occurring at the age of 34. Genetic analysis revealed a nucleotide substitution, resulting in a proline to leucine amino acid substitution (L47P). The genotype-phenotype correlations are remarkably strong, with mutations causing increasingly severe clinical features in the following order: S49P, S52R, L47P, H338R, G392E and G392R (*table 1*). In general, increasing clinical severity is characterized by earlier onset of the symptom and an increasing contribution

from the epileptic component of the syndrome. More specifically, individuals with the S49P mutation have diffuse small intraneuronal inclusions of neuroserpin with an onset of dementia between the ages of 45 and 60 (Davis *et al.*, 1999a; Davis *et al.*, 1999b; Bradshaw *et al.*, 2001). People with the S52R, L47P and H338R variants have larger and more numerous intraneuronal inclusions associated with progressively earlier onset of symptoms, during early adulthood (S52R, L47P) and adolescence (H338R) (Hagen *et al.*, 2011; Miranda *et al.*, 2008). In the most severe cases, caused by the most polymerogenic mutations, namely G392R and G392E, the patients exhibit the earliest onset of symptoms, with profound intellectual decline during childhood associated with severe, uncontrolled epilepsy (Coutelier *et al.*, 2008). While FENIB is a rare disease, with only a few known kindreds worldwide, diagnosis should be considered in patients that present with a frontal-type dementia combined with epilepsy, particularly when eosinophilic inclusions are seen on brain biopsy or at post-mortem.

The pathological polymerisation of neuroserpin variants results in FENIB

These remarkable genotype-phenotype correlations that are evident in the clinic are mirrored by the biophysical and biochemical properties of the variant NS proteins. Under physiological conditions, the rate of aggregation of the least clinically-aggressive NS mutant, S49P, is more than 10 times higher than that of the wild type protein, whereas the association rate constant for tPA is essentially unchanged (Belorgey *et al.*, 2002). The next most severe mutation, S52R, results in a further tenfold increase in the polymerization rate and the loss of effective tPA inhibition (Belorgey *et al.*, 2004). Thereafter, the aggregation rate becomes so

high as to be practically immeasurable. In the classic model of serpin polymerisation, proposed by Carrell and Lomas (2002), the functional changes in NS are all caused by the progressive destabilization of the key structural element, termed β -sheet A, which forms the core of NS (*figure 1*). This structural perturbation favours the incorporation of the RCL from a second NS molecule over the physiological process of intramolecular RCL insertion that occurs during protease inhibition (Davis *et al.*, 2002). An initial loop-sheet polymerization event yields a NS dimer that retains a patent β -sheet A at one end and a destabilized RCL at the other. Such a dimer is competent to undergo further loop-sheet polymerization to form trimers and eventually higher-order polymers. Recently, Huntington and colleagues have proposed alternative mechanisms for polymerization that require a more significant domain swap between the serpins in a chain (Sado *et al.*, 2009; Huntington & Whisstock, 2010; Belorgey *et al.*, 2011; Singh & Jairajpuri, 2011; Yamasaki *et al.*, 2011). In either case, the NS polymers gradually become entangled in the neuronal endoplasmic reticulum (ER) and form inclusions, known as Collins bodies. This phenomenon of aggregation or 'polymerization' has been described in other serpins such as α_1 -antitrypsin where it results in hepatocyte inclusions and liver disease (Lomas *et al.*, 1992; Elliott *et al.*, 1996; Huntington *et al.*, 1999). The observation that FENIB was caused by NS mutations (S49P and H338R) that are structurally homologous to substitutions in α_1 -antitrypsin, which also lead to polymerization (S53F and H334D, respectively) (Lomas *et al.*, 1993), argues strongly in favour of a shared molecular mechanism. This was confirmed by the finding that the neuronal inclusion bodies of FENIB were formed by polymers of NS with identical morphology to the polymers of mutant α_1 -antitrypsin present in hepatocytes from a child with α_1 -antitrypsin deficiency-related cirrhosis (Davis *et al.*, 1999a; Carrell & Lomas, 2002).

Dissecting the pathological mechanisms of the dementing and epileptic components of FENIB

The ER-resident inclusions (Miranda *et al.*, 2004) in the neurones of individuals expressing NS variants are likely to represent the toxic gain-of-function that results in this dominantly-inherited neurodegenerative syndrome. Serpin polymerisation exerts a stress on the cell that differs in important ways from other proteinopathies. In most cases, misfolding of proteins in the ER results in activation of the unfolded protein response (UPR). However, the native-like structure of serpin polymers results in a distinct signalling pathway called the ER overload response (Davies *et al.*, 2009).

This ER overload response activates NF- κ B by a pathway that is dependent on raised cytoplasmic Ca^{2+} levels. ER-associated degradation or ERAD is involved in the degradation of mutant NS and may be able to degrade the polymers (Ying *et al.*, 2011), whereas autophagy is more important in the bulk turnover of both wild type and mutant NS (Kroeger *et al.*, 2009). In the presence of polymerogenic mutations in serpins, and with increasing age, these mechanisms are overwhelmed and retention of polymers in the ER leads to cell death. This is apparent in the fly model of FENIB where the accumulation of intracellular polymers is associated with locomotor deficits where there was a correlation between the severity of the behavioural deficits and the degree of polymer accumulation (Miranda *et al.*, 2008).

While the intraneuronal accumulation of NS inclusions may underpin the dementia seen in FENIB, there is evidence that other mechanisms may also contribute to the epileptic propensity of these patients. In particular, it is notable that epilepsy is rarely seen when normal levels of tPA inhibitory activity are present, as is the case for wild type and S49P NS. This has been recently highlighted in relation to stroke, where patients thrombolized with recombinant tPA present a higher level of seizures (Alvarez *et al.*, 2013). When tPA inhibition is lost (for example, for the S52R and 392 mutants) then epilepsy becomes the major clinical feature (Davis *et al.*, 2002). Notably, neuroserpin is able to dampen neuronal sensitivity to excitotoxic stimuli by regulating tPA activity (Yepes *et al.*, 2000; Yepes *et al.*, 2002; Wu *et al.*, 2010). This effect appears to be mediated by the tPA-mediated proteolytic cleavage of the NR1 subunit of the NMDA receptor that increases the functioning of this excitatory glutamate receptor (*figure 2*) (Nicole *et al.*, 2001; Fernandez-Monreal *et al.*, 2004; Samson *et al.*, 2008; Baron *et al.*, 2010).

Excess glutamatergic neurotransmission is also a potent cause of epilepsy (reviewed in Vincent & Mulle [2009]) and increased NMDA signalling in response to dysregulated tPA activity could contribute to FENIB. Indeed, co-injection of neuroserpin with kainate into the hippocampus of the mouse attenuated the spread of consequent epileptic activity when compared to co-injection of kainate with vehicle alone (Yepes *et al.*, 2002). These data suggest a role for tPA in seizure spread in epilepsy and support the use of NS or others tPA inhibitors as a potential therapy.

Conclusion

Familial encephalopathy with neuroserpin inclusion bodies is a recently described neurodegenerative disease that is responsible for progressive myoclonic epilepsy and/or pre-senile dementia. Serpinopathies

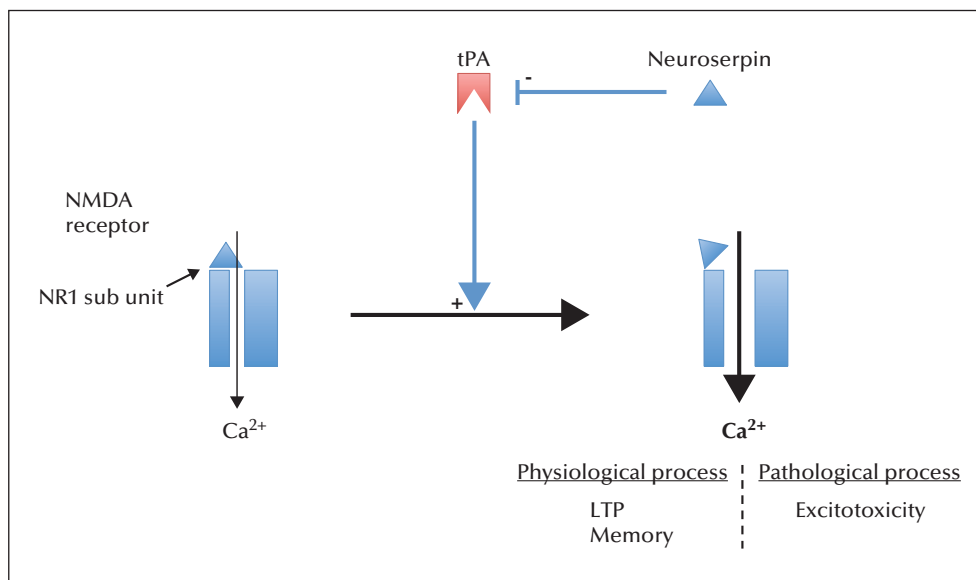


Figure 2. The NMDA receptor mediates the influx of calcium into neurones in the presence of glutamate. tPA has been shown to cleave the NR1 subunit (triangle) of the receptor and, as a consequence, increases the concentration of intracellular calcium. By inhibiting tPA, neuroserpin is thought to protect neurons from the toxic consequences of excessive NMDA activity, termed 'excitotoxicity'.

are unique among conformational diseases because they form native-like aggregates or polymers in the ER of synthetic cells. Serpin variants accumulate as inclusion bodies and thus activate the ER-overload response. It appears that this gain-of-function toxicity is responsible for the neuronal dysfunction and death that underpins dementia in FENIB. The evidence is less clear as to whether similar gain-of-toxicity is the cause of the epilepsy in FENIB or whether this results from dysfunction of the NS/tPA system. The relative importance of these two mechanisms has yet to be clearly elucidated. To date, genetic analysis of the *SERPINI1* gene should be performed in patients with adult-onset PME and early-onset, rapidly progressive cognitive dysfunction or predominantly frontal dementia. □

Disclosures.

None of the authors have any conflict of interest to disclose.

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