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Molecular Genetics and Transgenic Model of Gertsmann–Sträussler–Scheinker Disease

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*Karen Hsiao and *†Stanley B. Prusiner

*Departments of *Neurology and †Biochemistry and Biophysics, University of California, San Francisco, California, U.S.A.*

Summary: Gerstmann–Sträussler–Scheinker disease (GSS) is a rare, dominantly inherited neurodegenerative disease that can sometimes be transmitted to experimental animals through intracerebral inoculation of brain homogenates from patients. Substitution of leucine for proline at codon 102 of the prion protein gene has been found in several families with the disease; this mutation is genetically linked to GSS. Mice containing murine prion protein transgenes with this mutation spontaneously develop neurologic symptoms of ataxia, lethargy, and rigidity accompanied by spongiform degeneration throughout the brain. Thus, many of the clinical and pathological features of the GSS have been reproduced in this transgenic mouse paradigm; to our knowledge, this study illustrates, for the first time, that a neurodegenerative process similar to a human disease can be genetically modeled in animals. Whether or not this transgenic mouse model of GSS may facilitate the understanding of common neurodegenerative disorders such as Alzheimer disease remains to be established. **Key Words:** Prion—Creutzfeldt–Jakob disease—Gerstmann–Sträussler–Scheinker disease—Scrapie.

Gerstmann–Sträussler–Scheinker disease (GSS), also known as Sträussler's disease and Gerstmann–Sträussler syndrome, was first described by the Austrian neurologists J. Gerstmann and E. Sträussler and the neuropathologist I. Scheinker in 1936 (Gerstmann et al., 1936). It has been defined as a "spinocerebellar ataxia with dementia and plaque-like deposits" (Seitelberger, 1981). The original case of GSS and the majority of subsequently diagnosed cases were familial; about one-half of the members of each generation were affected. A few sporadic cases resembling GSS clinically and pathologically have been reported (Masters et al., 1981). Until recently, there has been discussion of the exact classification of GSS and diseases resembling GSS (Kuzuhara et al., 1983); however, the development

Address correspondence and reprint requests to Dr. K. Hsiao at the Department of Neurology, HSE-781, University of California, San Francisco, CA 94143-0518, U.S.A.

of new immunohistologic and molecular genetic markers has greatly improved our ability to diagnose GSS.

The clinical manifestations of GSS may vary, even within the same pedigree (Masters et al., 1981). Nevertheless, it is useful to examine a weighted composite of clinical features in several members of a given family; sometimes this approach has enabled investigators to decipher different clinical forms of GSS. In "ataxic GSS," patients typically complain of difficulty walking and unsteadiness, sometimes accompanied by leg pains or paresthesias in the early stages of disease. Later, mental and behavioral deterioration may occur. Examination usually reveals prominent cerebellar ataxia, dysarthria, ocular dysmetria, and hypo- or areflexia in the lower extremities with extensor plantar responses. Mild wasting or weakness and impairment of vibratory and proprioceptive sensations may be detected in the lower extremities. Dysphagia frequently develops and contributes to inanition in the final stages of disease. By contrast, in "dementing GSS," patients typically complain of cognitive and behavioral changes in the early stages of disease (Warter et al., 1982; Nochlin et al., 1989). Later, parkinsonism, pseudobulbar signs, and spasticity may develop. Ataxia is present in some cases.

The pathologic hallmark of GSS is the multicentric amyloid plaque that contains prion protein (PrP) (Kitamoto et al., 1986; Roberts et al., 1986; DeArmond et al., 1987). The amyloid plaques are deposited mainly in the cerebral and cerebellar hemispheres. The amyloid plaques of GSS specifically stain with polyclonal antisera raised against PrP 27–30 isolated from scrapie-infected hamster brains. The diagnosis of GSS is most effectively established when immunohistologic examination of formalin-fixed brain embedded in paraffin blocks reveals multicentric PrP plaques. White matter degeneration resembling that of other system degenerations, such as Friedreich's ataxia, is a prominent feature in most cases of GSS (Seitelberger, 1981). Neuronal loss occurs in scattered areas throughout the brain and spinal cord. Spongiform change is variable in degree and extent. Neurofibrillary tangles, if present, are usually observed in quantity and locations consistent with the age of the patient; however, in one family with GSS, they are visible in pathologic numbers (Azzarelli et al., 1985; Farlow et al., 1989; Ghetti et al., 1989).

GSS belongs to a group of related conditions, including Creutzfeldt–Jakob disease (CJD) and kuru, that are clinically, pathologically, and biochemically similar to scrapie, a neurodegenerative disease naturally found in sheep and goats. These diseases can be transmitted to experimental animals through intracerebral inoculation of brain homogenates from patients and animals with the disease. The transmissible agent, termed "prion" to distinguish it from viruses and viroids, has been found to be largely comprised of an aberrant form of a 33–35 kDa host glycoprotein, called PrP, and contains little or no nucleic acid (Prusiner, 1982). PrP is encoded in humans by a single-copy gene on chromosome 20 (Sparkes et al., 1986). The entire 253-codon open reading frame is located in one exon (Basler et al., 1986; Kretzschmar et al., 1986). A more extensive discussion of the biochemistry and genetics of prions can be found elsewhere (Prusiner, 1987,1991).

Assuming an autosomal-dominant mode of inheritance, genetic linkage analysis of a leucine for proline substitution at PrP codon 102 yielded a lod score of >3.0

in two unrelated Caucasian families (Hsiao et al., 1989*a*). This evidence is consistent with GSS being inherited despite also being infectious. An affected descendant of the first patient described by Gerstmann, Sträussler, and Scheinker was shown to have this PrP codon 102 leucine substitution (Kretszchmar et al., 1991). The PrP codon 102 leucine was also found in two Asian patients with GSS, thus supporting the hypothesis that the mutation is more than a linked genetic marker, possibly causing the disease (Doh-ura et al., 1989; Hsiao et al., 1989*b*). This and two other point mutations in the PrP open reading frame have been found to segregate with disease in families with GSS (Doh-ura et al., 1989; Hsiao et al., 1991*a*; Hsiao et al., manuscript in preparation). Two point mutations and several insertional mutations have been found to segregate with disease in families with CJD (Goldgaber et al., 1989; Owen et al., 1989; Goldfarb et al., 1990,1991; Brown et al., 1991; Hsiao et al., 1991*b*). There is a strong but not absolute correspondence of the various PrP mutations with different clinical and pathological phenotypes. The molecular basis for the pathogenic effects of these mutations is not understood. A more complete review of the molecular genetics of GSS and CJD can be found elsewhere (Hsiao and Prusiner, 1990; Brown et al., 1991).

Transgenic mice were created to test the biologic activity of the PrP leucine substitution linked to GSS. Spontaneous ataxia, rigidity, and lethargy with spongiform degeneration and gliosis developed in two of four lines of transgenic mice containing murine PrP genes with a leucine substitution at codon 101 (homologous to codon 102 in humans) (Hsiao et al., 1990; unpublished data). Little or no detectable protease-resistant PrP was seen on Western blots of brain homogenates from these transgenic mice. This finding dissociates the spongiform neuropathology and clinical illness from the presence of protease-resistant PrP, and indicates that the pathogenesis of disease in these transgenic mice is not the consequence of protease-resistant PrP accumulation, but rather is due to an as yet undefined abnormality in the metabolism of the mutant PrP molecules. Transmission studies using brain homogenates from these transgenic mice are in progress. Preliminary data suggest that there may be a low level of infectivity. These results indicate that the clinical and some of the pathologic features of GSS may be reproduced in a transgenic mouse paradigm.

Our transgenic mouse studies differ sharply from attempts to model human familial amyloidotic polyneuropathy (FAP) type 1 in transgenic mice. Human FAP type 1 is an autosomal-dominantly inherited disease characterized by the onset of peripheral neuropathy followed by systemic amyloidosis in adults, usually between the ages of 25 and 40 years (Nakazato et al., 1987). The pathologic hallmark of FAP is the deposition of amyloid composed of transthyretin molecules in the peripheral nerves. At 24 months of age, transgenic mice expressing mutant human transthyretin genes showed no clinical symptoms but, when killed, showed considerable amyloid deposition in the kidneys, and less in other organs (Yi et al., 1991). These mice exhibited no clinical or pathologic evidence of amyloid polyneuropathy. Attempts to enhance amyloid deposition in these transgenic mice by repeated lipopolysaccharide injections were unsuccessful (Murakami et al., 1991). It is not understood why neuropathy fails to develop

in these transgenic mice, even though it precedes systemic amyloidosis in humans with FAP type 1.

By contrast, our transgenic mouse model of GSS recreates the clinical and many of the neuropathological features of GSS in the correct target organ. This occurs in the absence of detectable amyloid formation, and therefore throws into question the role of amyloid in the pathogenesis of this and perhaps other prion diseases. The development of central nervous system dysfunction in the absence of amyloidosis in transgenic mice that express PrP with the GSS mutation is consistent with earlier neuropathologic observations in humans and mice; brains from most patients with CJD and some mice with experimental scrapie are devoid of amyloid plaques (Masters and Richardson, 1978; McBride et al., 1988). Recent studies show that rod-shaped particles in purified scrapie prion preparations that exhibit the tinctorial properties of amyloid are only formed after limited proteolysis and detergent extraction (McKinley et al., 1991). There is no evidence that PrP amyloid is required for neurodegeneration in the prion diseases (Prusiner, 1991).

GSS is one of the rarest neurodegenerative diseases known, affecting roughly 2–5 individuals per 100 million. GSS has been confused with Huntington chorea, spinocerebellar degeneration, Alzheimer disease, and multiple sclerosis. Lately, growing awareness of GSS as a disease entity in combination with immunohistological and molecular genetic diagnostic techniques has led some investigators to propose that the actual prevalence of GSS and related conditions may be higher than previously estimates (Collinge et al., 1990).

A frequently raised question is why a disease as rare as GSS merits study at all. Although Alzheimer disease is >10,000 times more common than GSS, the two diseases are clinically similar: (a) they are both slowly progressive degenerative diseases of the central nervous system that manifest as dementia; (b) they generally afflict middle-aged and elderly individuals; and (c) they may occur as autosomal-dominant genetic disorders, but otherwise appear sporadically. There are some compelling pathologic similarities: (a) dystrophic axons and dendrites surrounding focal amyloid deposits, termed argyrophillic amyloid plaques, are found in both diseases; (b) heparan sulfate proteoglycans are found in association with plaques in both diseases (Snow et al., 1988; Snow et al., 1990); and (c) pathologic quantities of neurofibrillary tangles, while more consistently observed in AD, have also been observed in at least one family with GSS. Because of these clinical and pathologic similarities, the two diagnoses may be confused; at least two families formerly given diagnoses of Alzheimer disease have actually had GSS (Azzarelli et al., 1985; Farlow et al., 1989; Ghetti et al., 1989; Nochlin et al., 1989).

The amyloid in Alzheimer disease is comprised largely of the β-amyloid peptide (β-A4) (Glenner and Wong, 1984; Masters et al., 1985), consisting of 40–43 residues. β-A4 is derived from the β-amyloid precursor protein (β-APP) that through alternative splicing of exons may exist in several different forms, each containing approximately 700 amino acids (Goldgaber et al., 1987; Kang et al., 1987; Tanzi et al., 1987*a*). Synthetic peptides corresponding to the sequence of β-A4 polymerize spontaneously in filamentous structures with the tinctorial properties of amyloid (Kirschner et al., 1987). Recent work suggests that β-A4 is

generated from β-APP by limited proteolysis involving the failure of a protease to cleave within the β-A4 region of β-APP (Esch et al., 1990; Sisodia et al., 1990; Holtzman and Mobley, 1991); phosphorylation of β-APP may also regulate its degradation (Buxbaum et al., 1990). The amyloid in prion diseases is composed largely of PrP molecules that, like β-A4, polymerize into filamentous structures. Limited proteolysis of PrP^{Sc} generates PrP 27–30, which assembles into amyloid rods in the presence of detergent (McKinley et al., 1991). Sequencing of protein from amyloid plaques isolated from brains of patients dying of GSS demonstrates an 11 kDa fragment of PrP containing amino acid residues 58 to 150 (Tagliavini et al., 1991). A number of comparisons between β-APP and PrP further extend the molecular parallels between the amyloid deposits in Alzheimer disease and those in prion diseases; (a) both are membrane-associated glycoproteins expressed predominantly in the brain (Bolton et al., 1982; Prusiner, 1982; Dyrks et al., 1988; Selkoe et al., 1988); (b) all of PrP^{C} and >90% of β-APP are located on the external surface of the plasma membrane (Stahl et al., 1987; Dyrks et al., 1988); (c) the promoter regions of the genes encoding both proteins lack TATA boxes and contain GC-rich regions characteristic for "housekeeping" genes (Basler et al., 1986; Salbaum et al., 1988); (d) both may be expressed as integral membrane and secretory proteins in vitro (Hay et al., 1987*a*,*b*; Dyrks et al., 1988); and (e) genetic linkage analyses have revealed disease loci on the same chromosomes as the amyloid protein genes for some forms of disease (St. George-Hyslop et al., 1987; Van Broeckhoven et al., 1988; Goate et al., 1989; Hsiao et al., 1989*a*).

Genetic modelling of GSS in transgenic mice has demonstrated that the PrP gene is the GSS disease locus (Hsiao et al., 1990). The β-APP gene is tightly linked to congophilic angiopathy in Dutch families (Van Broeckhoven et al., 1990) and a point mutation in β-A4 has been identified in some family members (Levy et al., 1990). A point mutation in β-A4 has been identified in two Caucasian and two Asian families with AD (Goate et al., 1991; Naruse et al., 1991). The β-APP gene may be the disease locus for Dutch congophilic angiopathy and some cases of AD.

Recombination between the β-APP gene and Alzheimer disease has been observed, thus excluding β-APP as the disease locus in other families (Tanzi et al., 1987*b*). Familial Alzheimer disease is probably not a single homogeneous genetic disorder (Schellenberg et al., 1988; St. George-Hyslop et al., 1990). It is possible that genetic loci outside the PrP open reading frame can also provoke prion illnesses, but there is currently no evidence to support this hypothesis.

In recent years, an impressive body of knowledge about the molecular pathology of Alzheimer disease has been accumulated, but no coherent, focused theory has emerged to provide an etiology for the disease. This stems largely from the absence of a convincing animal model. By contrast, a wide variety of independent disciplines including protein chemistry, molecular genetics, immunochemistry, neuropathology, and experimental neurology have greatly strengthened the hypothesis that PrP plays a central role in the etiology of human and animal prion diseases. The crux of the prion hypothesis will be established when the chemical mechanisms responsible for the conversion of the normal cellular isoform of PrP or a precursor into the abnormal disease-associated isoform are elucidated. Because benign, cellular proteins are converted into aberrant isoforms

in Alzheimer disease, some of the pathogenic processes in GSS may provide new and crucial insights into Alzheimer's disease. The transgenic model of GSS may permit the study of how environmental and other genetic factors can influence the onset and pathogenesis of disease. Hopefully, new knowledge about GSS will lead to effective therapies in a variety of neurodegenerative disorders.

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