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Case report

# Peripheral nerve hyperexcitability with preterminal nerve and neuromuscular junction remodeling is a hallmark of Schwartz-Jampel syndrome

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**Abstract**

Schwartz-Jampel syndrome (SJS) is a recessive disorder with muscle hyperactivity that results from hypomorphic mutations in the perlecan gene, a basement membrane proteoglycan. Analyses done on a mouse model have suggested that SJS is a congenital form of distal peripheral nerve hyperexcitability resulting from synaptic acetylcholinesterase deficiency, nerve terminal instability with preterminal amyelination, and subtle peripheral nerve changes. We investigated one adult patient with SJS to study this statement in humans. Perlecan deficiency due to hypomorphic mutations was observed in the patient biological samples. Electroneuromyography showed normal nerve conduction, neuromuscular transmission, and compound nerve action potentials while multiple measures of peripheral nerve excitability along the nerve trunk did not detect changes. Needle electromyography detected complex repetitive discharges without any evidence for neuromuscular transmission failure. The study of muscle biopsies containing neuromuscular junctions showed well-formed post-synaptic element, synaptic acetylcholinesterase deficiency, denervation of synaptic gutters with reinnervation by terminal sprouting, and long nonmyelinated preterminal nerve segments. These data support the notion of peripheral nerve hyperexcitability in SJS, which would originate distally from synergistic actions of peripheral nerve and neuromuscular junction changes as a result of perlecan deficiency.


*Keywords:* Schwartz-Jampel syndrome; Muscle stiffness; Peripheral nerve hyperexcitability; Basement membrane; Perlecan; Neuromuscular junction

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## 1. Introduction

Schwartz-Jampel syndrome (SJS) is a recessively inherited disorder characterized by permanent muscle stiffness with muscle hyperactivity detected at the electroneuromyographic examination, and chondrodysplasia [1]. The signs become obvious during the first 3 years of life in its classical form, and the disease course is considered to be slowly progressive until mid-adolescence and then to remain stable. The most recognizable feature is a "mask-like face" with narrow palpebral fissures due to blepharophimosis and blepharospasm, pursed lips, and reduced mobility of the facial muscles. Muscle size may be changed with either hypertrophy or atrophy. Muscle hyperactivity is linked to spontaneous activity on electromyogram (EMG) at rest. This spontaneous activity was a long subject of debate since typical myotonic discharges that wax and wane, as well as spontaneous sustained discharges that are sensitive to neuromuscular block and persist for long periods, were reported [2–5]. In addition to neuromuscular phenotype, patients display signs of primary chondrodysplasia with pectus carinatum, kyphoscoliosis, lumbar lordosis, bowing of the long bones, and slight dwarfism that may help to distinguish SJS from severe forms of myotonia due to mutations in the muscle sodium and chloride channels.

SJS results from hypomorphic mutations of the perlecan gene, including splicing, nonsense and missense mutations, and large genomic deletions [6]. A common feature of the SJS mutations is to induce a residual secretion of perlecan [7,8], which counteracts the lethality resulting from the complete lack of perlecan in mammals [9,10]. Perlecan is an extracellular heparan sulfate proteoglycan (HSPG) present in all basement membranes (BM) that plays a role in structural maintenance, cell attachment, and cell signalling [11]. Thus, the relationship between reduced amount of this ubiquitous HSPG and muscle stiffness was unclear. Perlecan is enriched at the neuromuscular junction (NMJ) where it is involved in the anchorage of acetylcholinesterase (AChE) by interacting with the ColQ collagen-like tail of this enzyme and the α subunit of the cell receptor dystroglycan [12]. Initial investigation done on mice knocked-out for perlecan suggested synaptic AChE deficiency as the cause of muscle hyperactivity in SJS [6,13]. However, this simple explanation was not so evident since repetitive stimulation upon AChE inactivation leads to transmission failure by AChR desensitization [14]. Accordingly, congenital AChE deficiency at the NMJ in Human is associated with myasthenia, i.e. muscle weakness and fatigability due to failure of neuromuscular transmission during repetitive activity [15].

The physiological investigations done on a mouse model of SJS, which has been obtained by introducing SJS-related mutations into the mouse perlecan gene, have suggested peripheral nerve hyperexcitability (PNH) without any major defect in neuromuscular transmission in SJS [16–18]. A combination of events occuring in this model has been proposed to induce PNH, including synaptic AChE deficiency, instability of the presynaptic axonal element leading to partial denervation of NMJs with long nonmyelinated preterminal segments, and focally persistent axonal depolarization. We report here the clinical, neurophysiological and myopathological findings in one patient with SJS in order to determine whether these conclusions are consistent with human pathophysiology.

## 2. Materials and methods

### 2.1. Biological samples

Genomic DNA was extracted from whole blood by standard phenol–chloroform procedure. Primary fibroblast cells were derived from punch skin biopsy. The muscle biopsy containing NMJs was taken by open biopsy of the left deltoid muscle. Patients without detectable neuromuscular defects were used as controls. The study was approved by the Ethics Committees of the involved institutions and was conducted with the informed consent of each participating individual.

### 2.2. Molecular and cellular analyses

The 97 exons of the perlecan gene were sequenced on genomic DNA as previously described [8]. Effect of the variants on RNA splicing was tested by calculating the consensus value of splice sites using the Human splicing finder matrix [19]. Total RNA was extracted from fibroblast cells using Trizol reagent (Life Technologies™, Saint-Aubin, France) and reverse transcribed using random hexamers according to the manufacturer's protocol (Thermoscript RT-PCR System, Life Technologies™). Semi-quantitative analyses were done on PCR products to determine the level of perlecan transcript compared to the level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) transcript. Amplicons were analyzed on 2% agarose gels stained with ethidium bromide. Gel pictures were scanned and the integrated density of each band was analyzed using ImageJ software (NIH, Bethesda, MD).

Primary fibroblast cells were maintained in DMEM medium with glutamax (Life Technologies™) supplemented with 10% fetal calf serum and 1% penicillin-streptamycin (Life Technologies™) in a 5% $CO_2$ atmosphere incubator at 37 °C. Fluorescent immunostaining analyses of fibroblast cells were conducted as previously described [8]. The amount of perlecan secreted by fibroblast cells was quantified by dot-blot analyses of conditioned culture media using fibronectin as control of loading as described [16].

### 2.3. Electroneuromyography (ENMG) and multiple measures of peripheral nerve excitability

The ENMG examination was done using standardized protocols [20]. Compound muscle action potentials (CMAP) were recorded using surface electrodes (belly-tendon technique) after supramaximal nerve