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Increased gene expression of growth associated protein-43 in skin of patients with early-stage peripheral neuropathies

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ABSTRACT

Growth associated protein-43 (GAP-43) is one of the neural proteins associated with nerve injury that is upregulated after nerve injury. To investigate whether GAP-43 quantification in skin biopsies would differentiate subtypes of peripheral neuropathies, we analyzed GAP-43 expression in skin from the lateral thigh and the distal leg. We prospectively enrolled 130 patients with peripheral neuropathies and compared data with healthy controls. Intraepidermal nerve fiber density (IENFD) was determined using antibodies against protein gene product 9.5 (PGP 9.5); anti-GAP-43 antibodies were applied to visualize regenerating nerve fibers. PGP 9.5 and GAP-43 gene expression was analyzed using qRT-PCR. Patients with neuropathies had a generalized reduction of IENFD and GAP-43 immunoreactive fibers compared to controls ($p < 0.01$). In contrast, cutaneous GAP-43 gene expression was increased in proximal skin in patients ($p < 0.05$), particularly when disease duration was short (<3 years; $p < 0.01$). While fiber density for both markers decreased with age in healthy skin ($p < 0.01$), age-dependent reduction of skin innervation was absent in neuropathies. Diagnostic subgroups and neuropathic pain had no influence on skin innervation. We conclude that peripheral neuropathies lead to an initial increase in GAP-43 gene expression as a potential mechanism of regeneration, which is not sustained in neuropathies of long duration.

1. Introduction

The assessment of skin innervation has become a useful tool in diagnosing peripheral neuropathies. Quantification of the intraepidermal nerve fiber density (IENFD) with the panaxonal marker protein–gene product 9.5 (PGP 9.5) can improve diagnostics especially when searching for small fiber involvement [1,2]. PGP 9.5 is a member of the ubiquitin hydrolase family (also called ubiquitin carboxyl terminal hydrolase-1, uch-l1) and a cytoplasmic protein expressed in neurons and in neuroendocrine cells [3]. Thus, when using PGP 9.5, all myelinated and unmyelinated nerve fibers are stained without differentiating nerve fiber subtypes.

Peripheral nerve lesion leads to an upregulation of growth factors and of regeneration-associated proteins including actin, tubulin, and growth associated protein-43 (GAP-43) [4]. GAP-43 is an axonal phosphoprotein that is expressed in the developing brain and in regenerating neurites as part of the neuronal growth cone [5,6]. Besides its role in the generation and modulation of synaptic connections in the central nervous system [7], GAP-43 is assumed to be crucial in axonal sprouting, growth, and regeneration via cytoskeletal reorganization in the peripheral nervous system. This may partly depend on its function as an osmosensory protein that augments intracellular calcium levels upon hypotonicity by release from inositol-3-phosphate-sensitive intracellular sources [8]. GAP-43 is therefore regarded as a marker for axonal regeneration [7,9]. Most peripheral neuropathies are characterized by concomitant de- and regeneration [10,11]. Given the role of GAP-43 in regenerative processes [12], its quantification may provide important information on the regenerative activity of nerve fibers. So far only few studies have investigated GAP-43 expression in skin biopsies, mostly focusing on diabetic neuropathy [13–17]. We set out to study GAP-43 immunoreactivity and gene expression in a large group of patients with peripheral neuropathies of different etiologies. We hypothesized that subgroups of neuropathies with potentially different regenerative capacities would differ in GAP-43 gene expression and immunoreactivity and that this difference might help predicting patients' outcome.

2. Subjects and methods

2.1. Patients and controls

Our study was approved by the Würzburg Medical Faculty Ethics Committee and written informed consent was obtained from every study participant before inclusion. We prospectively recruited 130 patients with peripheral neuropathies of initially unknown etiology who were cared for at our Department of Neurology, University of Würzburg, Germany between May 2007 and April 2010. The control group for skin

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punch biopsies consisted of 27 healthy volunteers without neurological diseases or pain. Patients reported for the diagnostic work-up of a suspected peripheral neuropathy or small fiber neuropathy (SFN); male and female patients ≥ 18 years were enrolled if no immunosuppressive or immunomodulatory treatment had been given before and if diagnostic skin punch biopsies had been obtained. Diagnosis of peripheral neuropathy based on patients' medical history, typical clinical symptoms and signs in the neurological examination including quantitative sensory testing according to the standardized German DFNS protocol ("Deutscher Forschungsverbund Neuropathischer Schmerz", German Research Network of Neuropathic pain) [18], on findings in neurophysiological assessment (see below), and on detailed laboratory data including: glucose metabolism parameters (HbA1c, oral glucose tolerance test), whole blood and differential cell counts, erythrocyte sedimentation rate, C-reactive protein, serum electrolytes, monoclonal immunoglobulins, vitamins B6 and B12, folic acid, renal and liver function tests, thyroid function tests, anti-nuclear antibodies (ANA), antibodies to extractable nuclear antigen (ENA), anti-neutrophil cytoplasmic autoantibody (ANCA), rheumatoid factor, serology of borreliosis and syphilis, immunofixation, and serum electrophoresis. Urine analysis was performed for Bence–Jones proteins, if the serum was positive for a monoclonal immunoglobulin. A complete neurophysiological assessment with standard nerve conduction studies in motor and sensory nerves at upper and lower limbs and needle electromyography in affected muscles was performed in all patients as part of the routine work-up as needed [19]. All patients also underwent diagnostic lumbar puncture, either at our department or they had undergone lumbar puncture in a hospital prior to admission to our clinic. The cerebrospinal fluid was tested for elevated white cell count and protein levels. If the underlying etiology could not be determined with these investigations a diagnostic sural nerve biopsy was performed following a standard procedure [20]. Table 1 shows the diagnostic subgroups and their definitions. The following diagnostic subgroups were distinguished:

Chronic inflammatory demyelinating neuropathy (CIDP): Patients were diagnosed as CIDP when the INCAT criteria were fulfilled (inflammatory neuropathy cause and treatment) [21].

"CIDPclin": These patients had the typical clinical presentation and laboratory findings, and showed a demyelinating neuropathy in neurophysiological and histological assessment as is characteristic for CIDP, but did not fulfill the neurophysiological INCAT criteria.

"CIDPsens": These patients had purely sensory symptoms with a duration of ≥2 months, signs of demyelination in neurophysiological assessment, signs of demyelination and inflammation in the sural nerve biopsy, elevated CSF protein, a positive response on steroid treatment, and normal to slightly reduced intraepidermal nerve fiber density (IENFD) in the skin punch biopsy taken from the distal lateral thigh and stained with the pan-axonal marker protein gene product 9.5 (PGP9.5, see below) [22,23].

Multifocal motor neuropathy (MMN), multifocal acquired demyelinating sensory and motor neuropathy (MADSAM), and Guillain–Barré syndrome (GBS): MMN, MADSAM, and GBS (in terms of an acute inflammatory and demyelinating polyneuropathy) were diagnosed according to published criteria [24–26].

Paraproteinemic neuropathies: Neuropathies with an IgM paraprotein with and without anti-MAG antibodies were summarized in this group [27].

Vasculitic neuropathy: These patients were divided in those with systemic vasculitis and those with non-systemic vasculitic neuropathy (NSVN) [28]

Diabetic neuropathy: Diabetic neuropathy was diagnosed if the patient had diabetes mellitus type I or II and if typical clinical, laboratory, and electrophysiological findings were present.

Hereditary neuropathy: Hereditary neuropathy was diagnosed if genetic testing was positive, or if clinical presentation, neurophysiological data, and family history were indicative.

Table 1
Diagnoses and diagnostic criteria.

Neuropathy	Abbreviation	Additional information	Reference
Chronic inflammatory demyelinating neuropathy	CIDP	According to the INCAT criteria (inflammatory neuropathy cause and treatment)	[21]
Chronic inflammatory demyelinating neuropathy-like clinical presentation	CIDPclin	Demyelinating neuropathy as defined by nerve conduction studies with typical clinical presentation, laboratory findings, and sural nerve pathology not fulfilling INCAT criteria	
Chronic inflammatory demyelinating neuropathy-like neuropathy, however, with pure sensory presentation	CIDPsens	Pure sensory clinical presentation, a symptom duration of ≥2 months, demyelinating features on nerve conduction studies, demyelination in sural nerve biopsy with small inflammatory infiltrates, elevated CSF protein, a positive effect upon steroid treatment, and normal to slightly reduced intraepidermal nerve fiber density in skin punch biopsy obtained from the distal lateral thigh	[22,23]
Neuropathy in systemic vasculitis and non-systemic vasculitis	NSVN		[28]
Multifocal motor neuropathy	MMN		[24]
Multifocal acquired demyelinating sensory and motor neuropathy	MADSAM		[25]
Guillain–Barré syndrome	GBS		[26]
Paraproteinemic neuropathies		Patients with a monoclonal IgM with or without anti-MAG antibodies	[27]
Hereditary neuropathy		Patients with a positive genetic test or if clinical presentation, neurophysiological data, and family history were suggestive of a hereditary type of neuropathy	
Diabetic neuropathy, type I or II		Patients with diabetes mellitus type I or II and if typical clinical, laboratory, and abnormal electrophysiological findings were present	
Other origin		Cases of definite other etiology such as neuropathy due to e.g. amyloidosis or paraneoplastic neuropathy	
Unknown etiology		Patients in whom a definitive diagnosis as detailed above was not possible at the time point of examination	
Idiopathic small fiber neuropathy	SFN		[29]

Abbreviations: CIDP: chronic inflammatory demyelinating polyneuropathy; CIDPclin: patients with a clinical presentation typical for CIDP, however, not fulfilling the neurophysiological INCAT criteria; CIDPsens: pure sensory clinical presentation; GBS: Guillain–Barré-syndrome; INCAT: Inflammatory Neuropathy Cause and Treatment Group; MADSAM: multifocal acquired demyelinating sensory and motor neuropathy; MMN: multifocal motor neuropathy; NSVN: non-systemic vasculitic neuropathy; SFN: small fiber neuropathy.