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Clinical Characterization of Sensory Neuropathy with Anti-FGFR3 Autoantibodies

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ABSTRACT

Objective Sensory neuropathies (SN) are often classified as idiopathic even if immunological mechanisms can be suspected. Antibodies against the intracellular domain of the Fibroblast Growth Factor Receptor 3 (FGFR3) possibly identify a subgroup of SN affecting mostly the dorsal root ganglion (DRG). The aim of this study was to identify the frequency of anti-FGFR3 antibodies and the associated clinical pattern in a large cohort of patients with SN.

Methods A prospective, multicentric, European and Brazilian study included adults with pure SN. Serum anti-FGRF3 antibodies were analyzed by ELISA. Detailed clinical and paraclinical data were collected for each anti-FGFR3-positive patient and as control for anti-FGFR3-negative patients from the same centers ("center-matched").

Results Sixty-five patients out of 426 (15%) had anti-FGFR3 antibodies, which were the only identified autoimmune markers in 43 patients (66%). The neuropathy was non-length dependent in 89% and classified as sensory neuronopathy in 64%, non-length-dependent small fiber neuropathy in 17% and other neuropathy in 19%. Specific clinical features occurred after 5-6 years of evolution including frequent paresthesia, predominant clinical and electrophysiological involvement of the lower limbs, and a less frequent mixed large and small fiber involvement. Brazilians had a higher frequency of anti-FGFR3 antibodies than Europeans (36% vs 13%, $p<0.001$), and a more frequent asymmetrical distribution of symptoms (odd ratio: 169, 3.4-8424 95% CI).

Conclusions Anti-FGFR3 antibodies occur in a subgroup of SN probably predominantly affecting the DRG. Differences between Europeans and Brazilians could suggest involvement of genetic or environmental factors.

INTRODUCTION

Sensory neurons of the peripheral nervous system are the target of many disorders including toxic, genetic, viral, and inflammatory diseases.[1] However, in about half of the patients with sensory neuropathy (SN), no cause is found after extensive workup.[2] Depending on the targeted population of neurons, patients develop ataxia, painful small fiber neuropathy (SFN), or a mixed painful and ataxic disorder. Disorders affecting the sensory neuron cell body in the dorsal root ganglia (DRG) are called sensory neuronopathies (SNN). A subgroup of sensory neuron diseases are immune mediated, such as paraneoplastic SNN or SNN/SFN associated with Sjögren syndrome (SS), and probably an unknown proportion of idiopathic cases.[1] Anti-Hu and anti-CV2/collapsin response mediator protein 5 (CRMP5) antibodies are highly specific of paraneoplastic SNN[3] while until recently, no antibodies were known in the other conditions. We have reported that an antibody reacting with the intracellular domain of the Fibroblast Growth Factor Receptor 3 (FGFR3) identifies a subgroup of patients with SN that mostly fulfilled the criteria of SNN.[4] Interestingly, in 60% of the patients with anti-FGFR3 antibodies, these antibodies were the only sign of an autoimmune context. However, this study resulted from a mono-center and retrospective analysis of only 16 anti-FGFR3-positive cases out of 106 SN patients. Here we report the results of a prospective, comparative, multicentric, and international study on 65 anti-FGFR3-positive patients out of 426 included SN revealing the pattern of the neuropathy. We also show that the frequency of anti-FGFR3 antibodies may be different in Europe and Brazil.

METHODS

Standard protocol approvals, registrations, and patient consents

The study was approved by the ethical committee of the University Hospital of Saint-Etienne (Clinical trial #NCT02539329) and by the committee for the protection of persons (n°2014-27). All participants provided written informed consent.

Study design and patients

Between February 3, 2015, and June 14, 2018, we conducted a prospective multicentric international study in 36 neuromuscular centers in Europe (France, Switzerland, and UK) and one in Brazil. The aim was to include at least 50 patients with anti-FGFR3 antibodies and 50 paired negative controls over 18 years of age and a clinically pure SN. Because we previously found that anti-FGFR3 antibodies may occur in some patients not fulfilling the criteria of SNN,[4] the present study included SNN, SFN, sensory chronic inflammatory demyelinating polyneuropathy (CIDP), length-dependent neuropathy (LDN) and other SN. All the patients should have a significant development of their neuropathy before entry in the study. Exclusion criteria were clinical motor involvement, neuropathies with established etiological diagnosis (diabetic, genetic, or toxic origin), or neuropathies associated with known autoantibodies (onconeural, anti-myelin-associated glycoprotein, and anti-ganglioside antibodies). The patients were investigated using conventional methods with neurological clinical examination and paraclinical investigations including at least electroneuromyography (ENMG) and a biological workup with autoantibody testing (table 1). Inclusion in the study required the availability of serum and a form collecting the SNN score, the final diagnosis retained by the neurologist following the patient in the center, and information about the autoimmune context of the neuropathy (table 1). A second form was sent to the clinicians to collect detailed clinical and paraclinical data for each anti-FGFR3-positive patient (table 1) and as control for one randomly chosen anti-FGFR3-negative patient from the same center ("center-matched"). We used this procedure of matching to characterize the anti-FGFR3-positive patients in terms of age, sex, and type of neuropathies and thus avoid selection bias related to the type of patients recruited in each center. This second form collected information on symptoms and signs, modified Rankin (M-Rankin) score, ENMG, skin biopsy or laser evoked potentials for SFN, biological workup, associated diseases, treatment, and appreciation of its effect according to the clinician's opinion (table 1). Two evaluations were requested at recruitment. The first described the status of the patient at full development of the neuropathy and the second retrospectively collected data concerning the neuropathy onset. A third evaluation obtained at least 6 months later was left to the discretion of the investigators. The final diagnosis of the neuropathy as defined by referring physicians was checked against the diagnostic criteria of SNN,[5] CIDP,[6] and SFN[7] by the authors JCA and JPC.

Table 1 Description of the data requested in the study forms

Data type	Requested fields
For all included patients (mandatory form)	Demographic data, verification of exclusion and inclusion criteria, SNN score, final retained diagnosis (SNN, CIDP, SFN, LDN, or other), autoimmune context (SS, SLE, IBD, or inflammatory rheumatism)
For anti-FGFR3-positive patients and "center-matched" negative patients (detailed form)	
Clinical data at onset	Date, course of neuropathy, sensory symptoms, topography of symptoms (UL, LL, proximal or distal)
Clinical data at the first clinical exam and at the last clinical exam	Date, symptoms as reported by the patient, distribution of sensory manifestations as reported by the patient and as observed on clinical examination (UL, LL, proximal or distal), presence of dysautonomia and/or ataxia, tendon reflexes, M-Rankin score and other clinical scores
ENMG	Date, copy of the reports, normal values of the ENMG laboratory
Paraclinical data	Associated diseases: diabetes, cancers, IBD, celiac disease, autoimmune hepatitis, inflammatory rheumatic disease, SLE, SS, Sharp syndrome, sclerodermia, sarcoidosis, other diseases Laboratory investigations: blood biochemical analyses (glucose, CRP, HbA1c, creatinine, liver enzymes, TSH, protein electrophoresis), immunology (complement, research of autoantibodies: onconeuronal, antineuronal, anti-ganglioside, ANA, anti-dsDNA, anti-SSA/SSB, ANCA, anti-citrulline, anti-mitochondrial, anti-tissue transglutaminase, rheumatoid factor, cryoglobulins) and hematology (CBC), cerebrospinal fluid analyses (total protein, glucose), biopsies (nerve, skin, or other) Imaging examination: PETscan, MRI, or other
Immunomodulatory treatment	Type, date of onset and end, posology, treatment efficiency (stabilization, failure, improvement, not clear)

ANA, antinuclear antibody; ANCA, antineutrophil cytoplasmic antibodies; anti-dsDNA, anti-double stranded DNA antibodies; CBC, complete blood count; CIDP, chronic inflammatory demyelinating polyneuropathy; CRP, C-reactive protein; IBD, inflammatory bowel disease; ENMG, electroneuromyography; HbA1c, glycosylated hemoglobin A1c; LDN, length-dependent neuropathy; LL, lower limbs; M-Rankin, modified-Rankin; MRI, Magnetic resonance imaging; Pet-scan**,** positron emission tomography; SFN, small fiber neuropathy; SLE, Systemic lupus erythematous; SNN, sensory neuronopathy; SS, Sjögren syndrome; SSA, Sjögren syndrome A; SSB Sjögren syndrome B; SNN, sensory neuronopathy; TSH, thyroid-stimulating hormone; UL, upper limbs.

Interpretation of electroneuromyography data

ENMG were performed according to the usual recommendations in current practice.[8-9] Motor and sensory conduction velocities were analyzed according to the normal values of each laboratory and expressed as a percentage of the lower limits of the normal (LLN). Amplitudes of sensory nerve action potentials (SNAPs) for median, ulnar, radial, superficial peroneal, and sural nerves were collected. The compound muscle action potentials (CMAPs) and motor conduction velocities (MCVs) between the wrist and the elbow were recorded in the median and ulnar nerve and between the ankle and knee for the tibial and peroneal nerves. The number of normal, abolished, and reduced SNAPs was calculated. For each motor nerve, three abnormal patterns were considered, as previously published:[10] a demyelinating pattern, an axonal pattern, and a mixed axonal/demyelinating pattern (see online supplementary table S1).

Detection of serum anti-FGFR3 antibodies and IgG isotypes

Detection of anti-FGFR3 antibodies was performed by using a previously described indirect ELISA.[11] We used the human recombinant intracellular domain of FGFR3 (Invitrogen, Carlsbad, California, USA) for the coating. For each serum, the signals were normalized for the serum-specific background noise by calculating the difference between the optical density (OD) of the FGFR3-coated well and that of the non-coated well (see supplementary methods S1). The test was considered positive when the difference was 3 standard deviations (SD) above the average signal of 58 apparently healthy blood donors (z-score >3). To determine IgG isotypes, indirect ELISA was repeated with secondary anti-human antibodies specific against IgG1, IgG2, IgG3, or IgG4 (see supplementary methods S1).

Statistical analysis

Categorical data were analyzed by the χ^2 test and continuous data by the Mann-Whitney test or Student's t-test depending on their distribution (Kolmogorov-Smirnov test). Quantitative results were expressed as median (interquartile range) or mean ± SD and categorical data were presented as count (percentage). A p-value of ≤ 0.05 was used to determine statistical significance after Bonferroni correction for multiple comparisons. Logistic regression adjusted for age and sex was used to characterize the clinical and paraclinical data of anti-FGFR3-positive patients. Parameters with p-value <0.2 in univariate analysis were entered into the logistic regression model by backward and forward method. Age and sex were introduced afterwards in the logistic regression together with the significant parameters of the first model. Data were expressed as odd ratio (OR), 95% confident interval (CI). To evaluate the logistic regression model we used a receiver operating characteristic (ROC) curve analysis. Missing data were excluded from the analysis both in uni- and multivariate analysis. Statistical analysis was performed with MedCalc Statistical Software version 18.2.1 (MedCalc Software, Ostend, Belgium).

RESULTS

Included population and screening of anti-FGFR3 antibodies

Four hundred and thirty-five patients were enrolled, 393 (90.3%) from European centers and 42 (9.7%) from Brazil. Of those enrolled, 9 did not undergo serum analysis due to exclusion criteria (figure 1), yielding 426 patients with anti-FGFR3 antibody results. Of these 426 patients, the diagnostic repartition was 240 SNN, 86 SFN, 31 CIDP, 25 LDN, and 15 other neuropathies (CIDP, LDN and other neuropathies were grouped as "other SN" [OSN]). For 29 patients, no final diagnosis was communicated. Of these 426 patients, 65 patients (15.3%) had anti-FGFR3 antibodies (figure 2), thereof 40 women and 25 men (ratio 1.6). Median age of onset was 53 (40-64). Fifteen patients (23%) came from Brazil and 50 from Europe (figure 2). The median concentration of anti-FGFR3 antibodies was 6.6 (5.0–12.3) µg/mL with a range of values between 3.8 and 745.6 µg/mL. In terms of IgG subtypes, IgG1 was the most frequent followed by IgG4 (see supplementary figure S1). We analyzed a second sample of a later blood sampling for 38 patients (33 anti-FGFR3-negative and 5 anti-FGFR3-positive patients) and the conclusion about the presence or not of anti-FGFR3 antibodies was always the same (see supplementary figure S1).

The clinical characteristics of the 65 anti-FGFR3-positive patients

Recruitment for the study occurred after a median delay of 2.9 (1.0-6.0) years from neuropathy onset. Anti-FGFR3 antibodies were the only identified autoimmune markers in 43 patients (66%). Forty-two patients (64.6%) were classified as SNN of whom 40 (95.2%) fulfilled the SNN diagnostic criteria,[5] 11 (16.9%) had SFN of whom 9 (82%) with a non-length dependent distribution, and 12 (18.5%) had OSN which was non-length dependent in 9 (table 2). Among patients with OSN, 2 patients fulfilled the criteria of CIDP[6] (one of them also met those of SNN[5]); 3 patients had ENMG changes suggesting demyelination in one nerve but the criteria of CIDP were not fulfilled; 3 patients had sensory abnormalities limited to the lower limbs (LL) with abolished Achilles reflexes suggesting LDN; the last 4 patients could not be classified: 1 had a pure multifocal painful neuropathy with perivascular cuffs of lymphocytes on nerve biopsy and a non-length dependent fiber loss on skin biopsy; 2 patients had a severe ataxic neuropathy with normal motor conduction velocities and normal or subnormal SNAPs but abnormal sensory evoked potentials in the four limbs indicating a proximal peripheral nerve disorder; the last patient had diffuse areflexia, gait instability and normal sensory examination.

The neuropathy onset was mostly chronic (42/64, 66%) and limited to the LL (27/65, 42%) or similarly affected the four limbs (33/65, 51%) and the distribution was asymmetrical in one third of patients (table 2). At recruitment, the neuropathy was non-length dependent in 58 patients (89%). The disorder was restricted to the LL in 18 (28%) and asymmetrical in 25 (39%). Pain was reported in half of the cases. Ataxia was more frequent in the LL (39/65, 60%) than in the UL (25/65, 39%). Autonomic nervous system disorders were rare (11/65, 17%) and included constipation, bladder dysfunction, orthostatic hypotension, and abnormal pupil reaction indicating involvement of the sympathetic and parasympathetic system (table 2). A third evaluation was obtained in 53 patients (82%, figure 3A) with a median delay of 5.9 (3.9-11.3) years from onset. The disorder was limited to the LL in a minority of cases (14/53, 26%) with a global symmetrical distribution (32/53, 60%). Pain was observed in 29 patients (56%), LL ataxia in 38 patients (72%), and dysautonomia in 15 patients (28%).

The ENMG recording was performed with a median delay of 3.7 (1.8-8.2) years after onset (table 2). In total, SNAPs were abnormal in the LL for 50/62 patients (81%) and in the UL for 22/62 (37%). Concerning motor conduction velocities, motor conduction velocities were strictly normal in the four limbs in 33% of patients (19/58), in the UL in 71% of patients (41/58), and in the LL in 47% (29/62). Cerebrospinal fluid (CSF) was analyzed in 40 patients and total CSF protein levels were elevated in 7 cases (18%).

Thirty-five patients (54%) received immune-modulatory treatments. Multivariate logistic regression found no treatment associated with improvement or stabilization, while steroids (OR 12.11 [0.9-160.0]) or intravenous (IV) Ig (OR 12.8 [1.2-141.8]) were significantly associated with deterioration.

Table 2 Clinical and paraclinical data of anti-FGFR3-positive patients at onset and at full development of the neuropathy (N=65)

Parameters	**Anti-FGFR3-positive patients**
Neurological diagnosis – N SNN/N SFN/ N OSN (% SNN)	42/11/12 (65%)
Clinical characteristics at the onset of neuropathy	
Age – median year (25^{th}-75^{th} percentile)	52.6 (39.7-64.0)
Sex - F/M (% of F)	40/25 (62%)
Course – N (%)	
Acute	9/64 (14%)
Subacute	13/64 (20%)
Progressive	42/64 (66%)
Symptoms reported by patient - N (%)	
Paresthesia/Dysesthesia	52/65 (80%)
Pain	35/65 (54%)
Ataxia	34/65 (52%)
Topography of reported symptoms - N (%)	
Purely LL	27/65 (42%)
Including UL	33/65 (51%)
Asymmetry	23/60 (38%)
Clinical characteristics at full development of the neuropathy	
Delay - median year (25^{th}-75^{th} percentile)	2.9 (1.0-6.0)
SNN score - median (25^{th}-75^{th} percentile)	9.9 (5.1-11.0)
Symptoms reported by patient - N (%)	
Paresthesia	51/65 (79%)
Dysesthesia	34/65 (52%)
Pain	36/65 (55%)
Topography of reported symptoms - N (%)	
Purely LL	18/65 (28%)
Including UL	46/65 (71%)
Asymmetry	25/65 (39%)
Clinical signs at clinical exam - N (%)	
Purely PTS altered (unmyelinated sensory fibers)	9/63 (14%)
Purely DSS altered (myelinated sensory fibers)	14/63 (22%)
PTS + DSS altered	38/63 (60%)
Face included	8/61 (13%)
Trunk included	9/62 (15%)
Ataxia UL	25/65 (39%)
Ataxia LL	39/65 (60%)
Global areflexia	18/65 (28%)
Dysautonomia	11/65 (17%)

M-Rankin – median (25th-75th percentile)	2.0 (1.0-2.0)
Associated autoimmune diseases – N (%)	22/65 (34%)
SS	10/64 (16%)
SLE	3/64 (5%)
AIH	2/64 (3%)
Other	7/64 (11%)
Other associated diseases - N (%)	
Cancer	3/64 (5%)
Electrophysiological data	
Delay - median year (25th-75th percentile)	3.7 (1.8-8.2)
Sensory nerve conduction - mean N ± SD (mean %)	
UL	
N tested nerves	5.0 ± 1.4
N abnormal SNAPs	3.9 ± 2.2 (77%)
N abolished SNAPs	1.5 ± 1.9 (30%)
LL	
N tested nerves	3.4 ± 0.9
N abnormal SNAPs	2.6 ± 1.5 (77%)
N abolished SNAPs	1.6 ± 1.6 (49%)
Motor nerve conduction (all limbs) - mean N ± SD (mean %)	
N tested nerves	5.8 ± 2.2
N nerves with normal pattern	4.7 ± 2.0 (75%)
N nerves with axonal pattern	0.6 ± 1.0 (8%)
N nerve with primary demyelinating pattern	0.2 ± 0.9 (4%)
N nerve with mixed axonal and demyelinating pattern	0.9 ± 1.3 (14%)

AIH: autoimmune hepatitis; DSS: Deep and superficial sensation; F: female; LL, lower limbs; LLN, lower limit of the normal; M: male; M-Rankin, modified-Rankin; N: number; OSN, other sensory neuropathies; PTS: pain and temperature sensation; SFN, small fiber neuropathy; SLE: Systemic lupus erythematous; SNAPs, sensory nerve action potentials; SNN: sensory neuronopathy; SS: Sjögren syndrome; UL, upper limbs.

Comparison of the neuropathy between anti-FGFR3-positive and anti-FGFR3-negative patients

To determine whether there exists a pattern of neuropathy specific to anti-FGFR3 antibodies, we compared the 65 anti-FGFR3-positive patients with the 66 center-matched anti-FGFR3-negative patients. The number of missing data was similar in the two groups. The median delays from onset to the first or the last clinical evaluation or ENMG recording were not different between the two groups (p=0.21, p=0.87, p=0.12). In univariate analysis (see supplementary table S2), the two populations did not show major differences regarding type of neuropathy, clinical manifestation at onset, first and last examination, associated diseases, and paraclinical investigations including ENMG. After Bonferroni correction, only age was significantly different, the anti-FGFR3-positive patients being older than controls (52.6 [39.7-64.0] vs. 48.0 [36.2-56.6] respectively, p=0.02). Multivariate logistic regression adjusted for age, sex, and for Brazilian origin found no difference in the two populations regarding the clinical and paraclinical data originating from the first examination. At the last examination, in contrast, patients with anti-FGFR3 antibodies had significantly more frequent paresthesia (OR 4.9, 1.2-19.3 95% CI, p=0.02), a higher number of abnormal SNAPs in LL (OR 1.7, 1.1-2.5 95% CI, p=0.01), a higher number of SNAPs >50% of the LLN in UL (OR 1.6, 1.0-2.6 95% CI, p=0.04), less frequent mixed alteration of myelinated and unmyelinated sensory fibers (OR 0.2, 0.07-0.77 95% CI, p=0.02), and less frequent trunk involvement (OR 0.2, 0.04-0.94 95% CI, p=0.04). The area under the curve (AUC) of the logistic regression model was 0.82 (0.73-0.89) suggesting that, with time, the clinical and paraclinical

pattern of the anti-FGFR3-positive patients became clearly distinct from that of the anti-FGFR3-negative one (table 3).

Table 3 Multivariate analysis by logistic regression with clinical and paraclinical data

Variables	OR (95% CI)	*P* value of the variable[a]	*P* value of the overall model[a]	AUC of ROC curve analysis (95% CI)
Anti-FGFR3-positive patients (N=65) vs. all anti-FGFR3-negative patients (N=361) (demographic and initial mandatory data)				
Age	1.03 (1.00-1.05)	**0.02**	**< 0.001**	0.68 (0.63-0.73)
Autoimmune context	1.36 (0.72-2.59)	0.34		
Country (Brazil)	7.3 (3.0-18.0)	**< 0.001**		
Sex (M)	0.89 (0.49-1.61)	0.70		
SNN score	0.93 (0.86-1.01)	0.10		
Anti-FGFR3-positive patients (N=65) vs. anti-FGFR3-negative patients (N=66) (Clinical data at last clinical exam)				
Age at onset	1.03 (0.99-1.08)	0.06	**< 0.001**	0.82 (0.73-0.89)
Sex (M)	0.64 (0.24-1.74)	0.39		
Country (Brazil)	3.89 (0.58-0.94)	0.16		
Topography: distal LL	3.65 (0.69-19.4)	0.13		
Topography: trunk	0.19 (0.04-0.94)	**0.04**		
Clinical signs: PTS and DSS alteration	0.22 (0.07-0.77)	**0.02**		
Symptoms: paresthesia	4.90 (1.24-19.3)	**0.02**		
N abnormal SNAPs >50% LLN in UL	1.63 (1.02-2.60)	**0.04**		
N abnormal SNAPs in LL	1.68 (1.11-2.54)	**0.01**		
Anti-FGFR3-positive Brazilian SNN patients (N=15) vs. anti-FGFR3-positive European SNN patients (N=27) (Clinical data at first exam)				
Age at onset	0.83 (0.68-1.01)	0.07	**< 0.001**	0.98 (0.87-0.99)
Sex (M)	0.57 (0.03-10.7)	0.70		
Topography: proximal UL	23.1 (1.3-409)	**0.03**		
Topography: asymmetry	169 (3.4-8424)	**0.01**		
Anti-FGFR3-positive Brazilian SNN patients (N=15) vs. anti-FGFR3-positive European SNN patients (N=27) (Clinical data at last exam)				
Age at onset	0.72 (0.54-0.96)	**0.02**	**< 0.001**	0.97 (0.85-0.99)
Sex (M)	2.04(0.14-29.3)	0.60		
Topography: asymmetry	1038 (5.4-197867)	**0.009**		

ANA, antinuclear antibody; AUC, area under the curve; DSS, deep and superficial sensation (myelinated sensory fibers); LL, lower limbs; LLN, lower limit of the normal; N, number; PTS, pain and temperature sensation (unmyelinated sensory fibers); ROC, Receiver operating characteristic; SNAPs, sensory nerve action potentials; SNN, sensory neuronopathy; UL, upper limbs.

[a] The bolded p-values are statistically significant (≤0.05).

Anti-FGFR3 antibodies in Brazilians and comparison of the neuropathy between Brazilian and European anti-FGFR3-positive patients

A comparison of the whole European and Brazilian population showed that 15 out of 42 (36%) Brazilian patients, all fulfilling the SNN criteria, had anti-FGFR3 antibodies against only 50 out of 381 (13%) European patients (p<0.001). Whatever the presence of anti-FGFR3 antibodies, Brazilians were younger than Europeans (40 [35-50] vs. 54 [42-63], p<0.001) and had more frequent autoimmune contexts (16/32 [50%] vs. 70/327 [21%], p<0.001) with a different distribution of their disorders (p<0.001). In detail, autoimmune hepatitis was only observed in Brazilians (n=4 cases) while SLE (6), inflammatory bowel-disease (2), rheumatoid arthritis (7),

uveitis (1), sarcoidosis (2), and Biermer's disease (2) were only present in the Europeans. Moreover, the frequency of SS was higher in Brazilians than in Europeans. Multivariate logistic regression showed that after adjustment for age, sex, SNN score, and presence of an associated autoimmune disease, a Brazilian status was still significantly associated with detection of anti-FGFR3 antibodies (OR: 7.3, 3.0-18.0 95% CI, p<0.001; table 3). The same results were obtained when comparing the frequency of anti-FGFR3 antibodies in Europeans and Brazilians with SNN only.

When comparing the detailed clinical and paraclinical data of the Brazilian (n=15) versus European (n=27) anti-FGFR3-positive SNN (table 4), we found the same differences in term of age and frequency of autoimmune disease as when comparing the whole populations. The clinical pattern of the SNN was different in the two populations with a more severe sensory impairment with time and a very different distribution of sensory disorders in Brazilians. At onset and during evolution, in Brazilians, sensory symptoms more frequently involved UL and LL proximally and were more frequently asymmetric comparatively to Europeans (figure 3B,C and table 4). In multivariate analysis (table 3), age of onset and asymmetrical distribution were significantly associated with a Brazilian origin. Comparatively to onset, the differences persisted at first clinical investigation and in addition, sensory symptoms and ataxia were more frequent in the UL in Brazilians (table 4). In multivariate analysis, a higher frequency of proximal involvement in UL and a higher asymmetrical distribution of symptoms were significantly associated with a Brazilian origin. At the last examination (figure 3B,C), the difference of symptoms distribution persisted and global areflexia and dysautonomia were more frequent in Brazilians (table 4). There was no difference concerning ENMG data.

Table 4 Significant clinical and paraclinical differences between the Brazilian (N=15) and European (N=27) SNN patients with anti-FGFR3 antibodies

Parameters	Brazilian Patients	European SNN Patients	*P* value[a]
Clinical characteristics at onset			
Age – median year (25th-75th percentile)	44 (39-50)	61 (50-68)	***< 0.001****
Sex - F/M (% of F)	11/4 (73%)	16/11 (59%)	0.37
Topography of reported symptoms - N (%)			
Proximal UL	10/15 (67%)	2/27 (7%)	***< 0.001****
Distal LL	3/15 (20%)	23/27 (85%)	***< 0.001****
Purely LL	0/15 (0%)	13/27 (48%)	***0.001****
Asymmetry	13/14 (93%)	6/25 (24%)	***< 0.001****
Clinical characteristics at the first clinical exam			
SNN Score – median (25th-75th percentile)	12.7 (10.5-12.7)	11.0 (8.5-11.0)	***0.009****
Topography of reported symptoms - N (%)			
Proximal UL	12/15 (80%)	4/27 (15%)	***< 0.001****
Asymmetry	14/15 (93%)	8/27 (30%)	***<0.001****
Clinical signs at clinical exam - N (%)			
Ataxia UL	11/15 (73%)	8/27 (30%)	***< 0.001****
Clinical characteristics at the last clinical exam			
Topography of reported symptoms - N (%)			
Distal LL	4/15 (27%)	21/23 (91%)	***< 0.001****
Proximal LL	11/15 (73%)	4/23 (17%)	***< 0.001****
Asymmetry	14/15 (93%)	6/23 (26%)	***< 0.001****
Clinical signs at clinical exam - N (%)			
Global areflexia	12/15 (80%)	6/23 (26%)	***0.001****
Dysautonomia	10/15 (67%)	2/23 (9%)	***< 0.001****

Associated autoimmune diseases - N (%)			
SS	7/15 (47%)	1/26 (4%)	***0.001****

F, female; LL, lower limbs; M, male; N, number; SNN, sensory neuronopathy; SS, Sjögren syndrome; UL, upper limbs.

[a] The p-values in bold and italic with the symbol "*" are statistically significant after Bonferroni-correction.

DISCUSSION

This prospective multicentric controlled study specifies the pattern of patients bearing anti-FGFR3 antibodies. Overall, 15% of the patients with SN enrolled in this study had anti-FGFR3 antibodies. About two-thirds of them fulfilled the diagnostic criteria of SNN, 17% had SFN, and 19% another form of SN. Interestingly the neuropathy had a non-length dependent distribution in about 89% of cases including SFN suggesting that the DRG were the main target of the disorder. This is supported by the electrophysiological study which shows that on average 77% of the SNAPs studied in a given patient were abnormal with 30% of them abolished in the UL. However, motor nerve conduction was abnormal in 26% of nerves in a given patient. The most frequent patterns were mild axonal or mild mixed axonal and demyelinating changes. Similar alterations are frequent in SNN as a whole [2] and more particularly in paraneoplastic SNN [12-13] or with SS.[14] The autonomic nervous system was involved in 28% of patients at the last clinical exam, another feature shared with paraneoplastic and SS-associated SNN.[15-16] Three patients fulfilled the ENMG criteria of a primary demyelinating disorder and were classified as CIDP by the referring clinician. Similarly, clearly demyelinating changes occasionally occur in patients with the onconeural anti-Hu and CV2/CRMP5 antibodies.[12-13] In only three patients, the distal part of sensory nerves in LL was affected both clinically and electrophysiologically. Another feature in patients with anti-FGFR3 antibodies, also shared with paraneoplastic[17-19] or SS-associated SNN[14] is a predominant involvement of large or small sensory neurons sometimes restricted to one or the other type,[20] explaining that pain or ataxia may prevail in some patients. These variations may depend on the fact that the expression of FGFRs including FGFR3 occurs both in large and small sensory neurons and is not restricted to their cell body since it is also present is axons and Schwann cells including non-myelinating Schwann cells.[21-25]

The clinical pattern of patients with anti-FGFR3 antibodies showed particular features, some of them already underlined in our previous study.[4] In detail, a mean age around 50 years, a high frequency of paresthesia, a less frequent mixed involvement of sensory myelinated and unmyelinated fibers, a more severe impairment of sensory conduction in LL, and a less impaired sensory conduction of UL characterize the anti-FGFR3 antibody-related neuropathy. Interestingly these features probably take several months or years to become apparent since they were statistically significant only at the last examination. This indicates a specific evolution of the neuropathy in patients with anti-FGFR3 antibodies which with time tends to be less severe in the UL than in the LL and predominate on either large proprioceptive or small pain related sensory neurons. These findings could justify the research of these auto-antibodies in routine practice.

Another important result is the high prevalence of anti-FGFR3 antibodies in the Brazilian population, almost three times that of Europeans. This difference applies regardless of whether we compare the two populations of neuropathies or only SNN in Europeans and Brazilians since Brazilians had SNN only, which is explained by the particular interest of the Brazilian center in this disorder. The Brazilian patients were younger than the Europeans and have more frequently an associated autoimmune disease. In addition, all of them had typical SNN. However, after

adjustment for these confounding factors, Brazilian origin was still highly associated with anti-FGFR3 antibodies. The clinical presentation of anti-FGFR3-positive SNN patients also differed between the Brazilian and European populations although the proportion of patients fulfilling the SNN diagnostic criteria was not different between the two populations. Thus, the distribution of sensory symptoms among the Brazilian anti-FGFR3-positive patients was more frequently asymmetrical, proximal, and affecting the UL. As the number of tested Brazilian patients is relatively limited, these results including the prevalence of anti-FGFR3 antibodies and the clinical pattern of the neuropathy need to be confirmed on a larger multicentric cohort.

Several factors may explain the higher prevalence of anti-FGFR3 antibodies in Brazil, among them environmental factors acquired early in life or a specific genetic background. The former may explain the younger age of onset and the latter the striking difference in the frequency and type of associated autoimmune diseases in the two populations and some differences in the pattern of the SNN. One particular difference about SNN with autoimmune context was the more frequent association of anti-FGFR3 antibodies with an underlying SS context in SNN patients from Brazil versus Europe although the same diagnostic criteria of SS were used by the different centers.[26] This could be explained by a possible higher overall incidence of SS in Brazil than in Europe or by a more common association of SNN with SS in Brazil than in Europe.[27]

Anti-FGFR3 antibodies were the only markers of an autoimmune reaction in 66% of cases. At present it is not possible to determine whether they play a pathogenic role or are only biomarkers of a dysimmune process as it is the case for example with most of the onconeural antibodies, particularly anti-Hu antibodies.[28-29] However, anti-FGFR3 antibodies recognize the intracellular domain of the protein [4] which contains the tyrosine kinase domains whose activation is responsible for signal transduction and regulation of many cell functions including survival.[30] Whether the antibody may enter the neurons and interfere with signal transduction remains at present speculative and needs further work. Anti-FGFR3 antibodies were mostly IgG1, associated or not with IgG3, as it is frequent in autoimmune diseases. These IgGs can activate the complement cascade or induce antibody-dependent toxicity by macrophages. Thirty percent of the patients had IgG4 which may have a quite different action by inducing functional blocking of receptor proteins.[31] However, the low number of identified cases does not allow any conclusion as whether IgG4 antibodies are associated with a specific clinical profile or response to treatment. In contrast to another publication,[32] we found the concentration of anti-FGFR3 antibodies to not differ significantly over time in anti-FGFR3-positive patients, but this needs to be confirmed in a larger number of cases.

This study was not designed to appreciate the effect of treatments on the neuropathy in patients with anti-FGFR3 antibodies. Only half of them received immunomodulatory treatments in an open uncontrolled way. Steroids or IVIg were associated with deterioration in anti-FGFR3-positive patients. However, one should keep in mind that many patients were probably treated too late in the evolution of their disease, while early treatment is crucial in these disorders to prevent irrevocable neuron cell death.[33]

ELISA was the technique used for antibody screening. The use of a single technique may potentially be a source of misclassification. For example, it is well-accepted that anti-myelin oligodendrocyte glycoprotein autoantibodies can be specifically detected via cell-based assays (CBA), but cause false positives in ELISA.[34] However, we have shown the concordance between ELISA and CBA in our previous study.[4] Furthermore, to reduce the risk of misclassification, we systematically added an antigen-free well to take SSBN into account, which is a major cause of false positive results in ELISA.[11]

In conclusion, our prospective, controlled, and international study confirms that anti-FGFR3 antibodies are associated with a subgroup of SN which mostly affects the DRG. Anti-FGFR3-positive neuropathies had clinical features that could help to distinguish them from other SN which can justify the research of these auto-antibodies in clinical practice. The prevalence of anti-FGFR3 antibodies appears to be higher in Brazilian compared to European patients, suggesting that environmental or genetic factors may lead to an autoimmune reaction against FGFR3.

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Contributors YT and JCA had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: JPC and JCA. Acquisition, analysis and interpretation of data: all authors. Drafting the manuscript: YT, CPM, JPC and JCA. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: YT and JCA.

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Patient consent for publication Obtained.

Ethics approval Ethical approval for the study was provided by the ethical committee of the University Hospital of Saint-Etienne (Clinical trial #NCT02539329) and by the committee for the protection of persons (n°2014-27). All participants provided written informed consent.

Data sharing statement Data are available on reasonable request.

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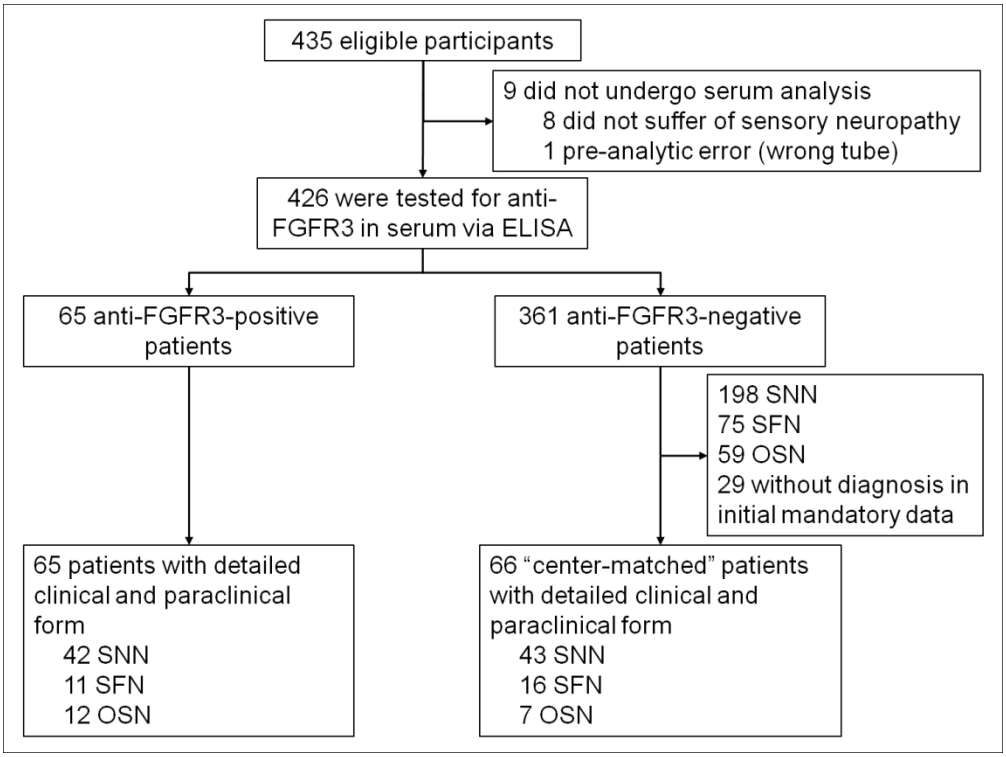
Figure titles and legends

Figure 1 Study profile. OSN, other sensory neuropathy; SFN, small fiber neuropathy; SNN, sensory neuronopathy.

Figure 2 Detection of anti-FGFR3 antibodies via indirect ELISA. The FGFR3-specific reactivity of each serum is represented by the optical density (OD) difference between FGFR3-coated wells and non-coated well (serum specific background noise normalization). A z-score ≥ 3 above the apparently healthy blood donors' mean value defines positivity (dotted line). These z-score ≥ 3 corresponds to an OD difference of ≥ 0.279 between FGFR3-coated wells and non-coated well. N, number; OSN, other sensory neuropathy; SFN, small fiber neuropathy; SNN, sensory neuronopathy.

Figure 3 Distribution of sensory symptoms and clinical signs at the last clinical exam in all anti-FGFR3-positive patients (A), and in European (B) and Brazilian (C) anti-FGFR3-positive SNN patients. The distribution of symptoms is indicated by the frequency of involvement and by a color code summarizing all the data (the darker the color, the higher the frequency of involvement). DSS, deep and superficial sensation (myelinated fibers); LL, lower limbs; N, number; Prox, proximal; PTS, pain and temperature sensation (unmyelinated fibers); UL, upper limbs.



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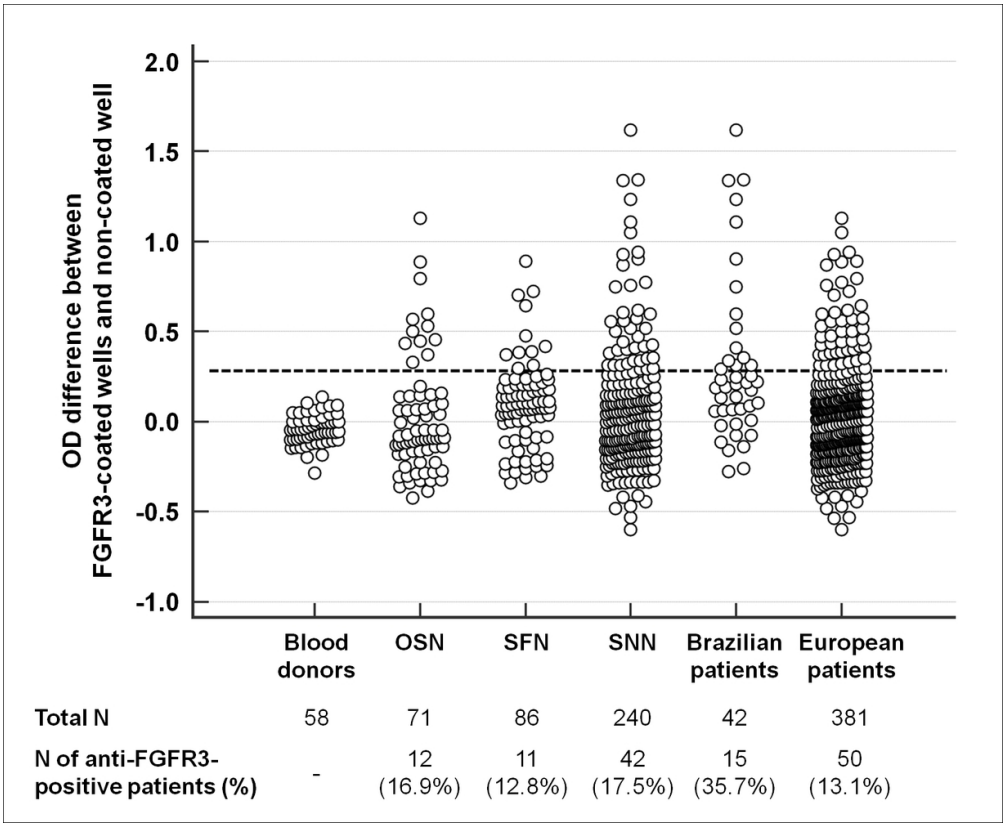

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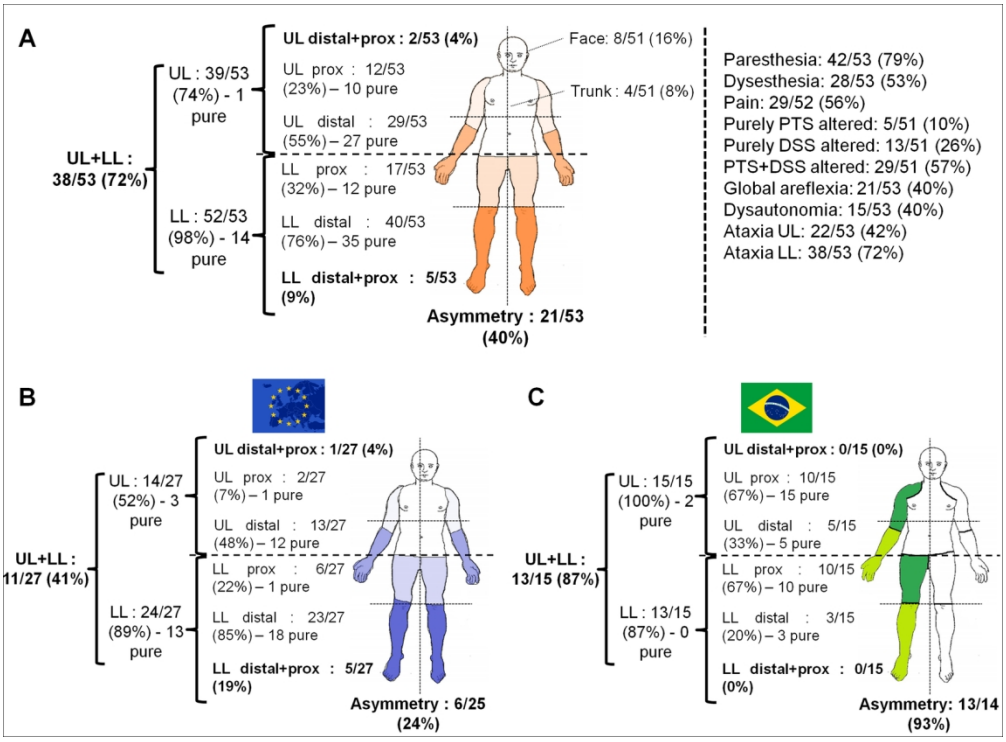

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148x108mm (300 x 300 DPI)

Supplementary table S1 Criteria for the definition of abnormal motor electrophysiological patterns

Demyelinating pattern	Axonal pattern	Mixed axonal/demyelinating pattern
MCV ≤80% LLN and CMAP ≥80% LLN	MCV >90% LLN and CMAP < LLN	MCV 80-90% LLN and CMAP 80-100% LLN
or	or	or
MCV ≤70% LLN and CMAP <80% LLN	MCV >80% LLN and CMAP <80% LLN	MCV <LLN without reaching values for demyelinating pattern and CMAP in the lower range of normal values
	or	
	Absence of CMAP despite several stimulations	

CMAP, compound motor action potential; LLN, lower limit of the normal; MCV, motor conduction velocity.

Supplementary methods S1

Detection of serum anti-FGFR3 antibodies by indirect ELISA

Maxisorb 96-well plates (Thermo Fisher Scientific, Waltham, Massachusetts, USA) were coated overnight at 4 °C with 5 µg/mL of the human recombinant intracellular domain of FGFR3 (Invitrogen, Carlsbad, California, USA) in carbonate/bicarbonate 0.05 M, pH 9.6 (Sigma-Aldrich, Saint-Louis, Missouri, USA). To quantify the serum-specific background noise (SSBN), a non-coated well was prepared for each sera-of-interest by incubating coating buffer without the FGFR3 protein. All wells were then blocked with 300 µL of 0.06% Tween-20, 0.1% fish gelatin, and 3% bovine serum albumin (BSA) in phosphate-buffered saline (PBS, Sigma-Aldrich), for 2h at room temperature (RT). The plates were after incubated overnight at 4°C with serum diluted samples (1:30 in blocking buffer). As a positive control, we used 1:3000 rabbit-anti-FGFR3 antibody (polyclonal Immunoglobulin (Ig) G, GeneTex, Irvine, USA). The plates were washed 7 times and incubated for 2h at 4°C with the appropriate peroxidase-conjugated secondary antibody solutions: 1:3000 of rabbit-anti-human IgG (Dako, Glostrup, Denmark) for human sera samples and 1:3000 of swine-anti-rabbit Ig (Dako) for the rabbit-anti-FGFR3 control antibody. The plates were then washed 10 times and incubated for 30 min with 0.4 mg/mL of O-phenylenediamine dihydrochloride in phosphate/citrate buffer (0.05 mM, pH 5, Sigma-Aldrich). Optical density (OD) was measured at 440 nm with Multiscan EX Elisa Reader (Thermo Fisher Scientific). The inter-assay (or inter-plates) variability of the ODs was evaluated by using three samples in each plate (two sera from healthy donors and the anti-FGFR3 antibody) and acceptability limits were defined as the OD mean ± 2 standard deviations (SD). In all experiments, the inter-assay coefficient of variation of OD was <10% for all three controls. Data normalization based on SSBN was realized for each serum: the difference between the OD of the FGFR3-coated well and that of the non-coated well was calculated and the test was considered positive when the difference was 3 SDs above the average apparently healthy blood donor (n=58) signal (z-score >3, corresponding to difference on OD scale >0.279). Blood donor sera were obtained via the “etablissement français du sang”. All positive patients’ sera were controlled in a second ELISA test to confirm the first result. The serum concentration of anti-FGFR3 antibody was estimated using calibration curve obtained with serial dilution of rabbit-anti-FGFR3 antibody.

Anti-FGFR3 IgG isotypes

To determine IgG isotypes of anti-FGFR3 antibodies, indirect ELISA was repeated with secondary horseradish peroxidase-conjugated anti-human antibodies specific against IgG1, IgG2, IgG3 (Life Technologies, Carlsbad, California, USA; 1:500 IgG1, 1:500 IgG2, 1:200) or IgG4 (abcam, Cambridge, United Kingdom; 1:350 IgG4). For each serum, the OD of FGFR3-coated wells were normalized as previously described. The test was considered positive when the difference was 3 SDs above the average of anti-FGFR3-negative patients (n=10) signal (z-score >3).

Supplementary table S2 Clinical and paraclinical data in anti-FGFR3-positive (N=65) and negative (N=66) patients

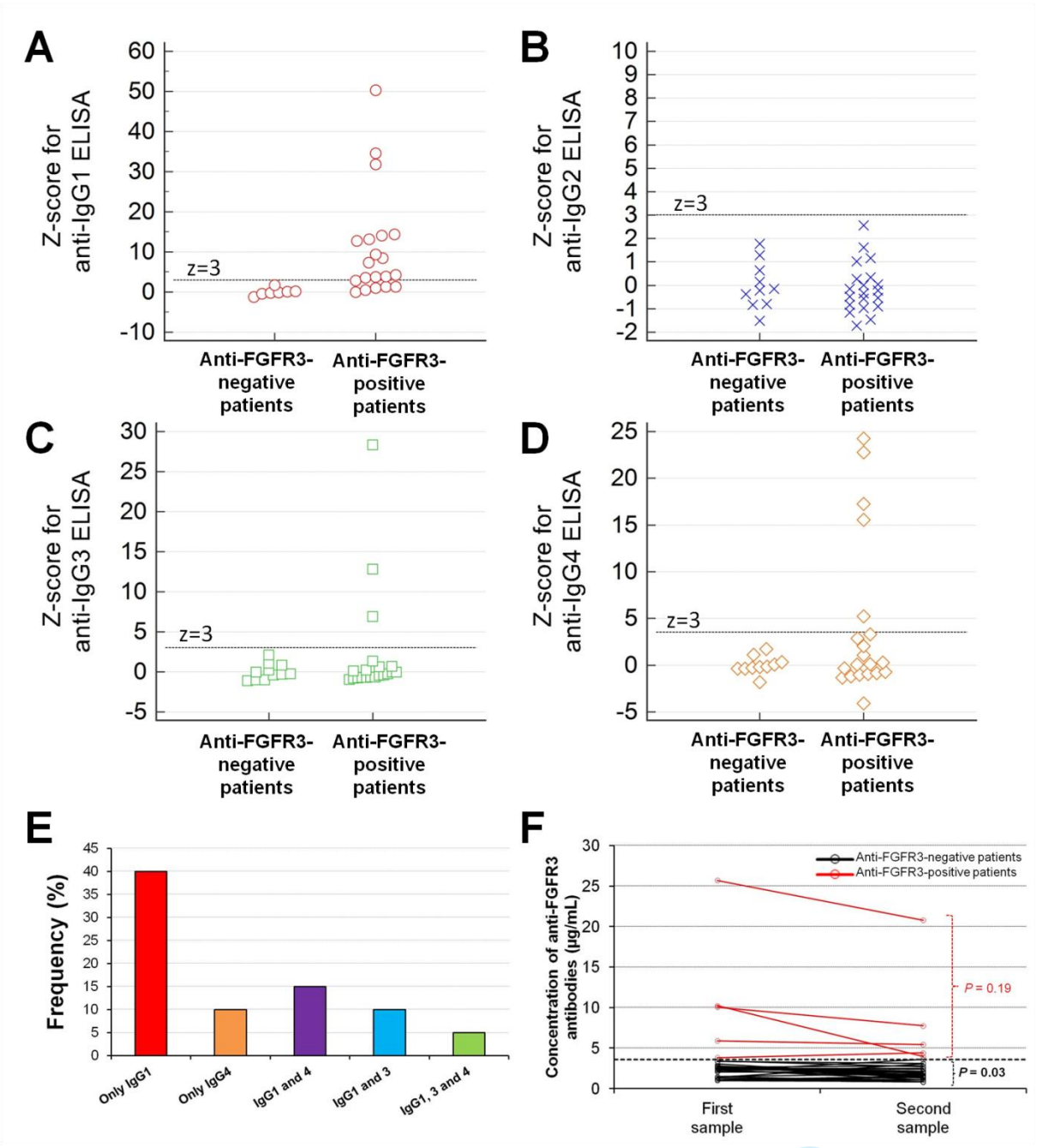
Parameters	Anti-FGFR3-positive patients	Anti-FGFR3-negative patients	p-value
Neurological diagnosis – N SNN/N SFN/ N OSN (% SNN)	42/11/12 (64.6%)	43/16/7 (65.2%)	0.33
Clinical characteristics at the onset of neuropathy			
Age – median year (25^{th}-75^{th} percentile)	52.6 (39.7-64.0)	48.0 (36.2-56.6)	***0.02****
Sex - F/M (% of F)	40/25 (61.5%)	37/29 (56.1%)	0.52
Course – N (%)			
Acute	9/64 (14.1%)	5/66 (7.6%)	0.23
Subacute	13/64 (20.3%)	11/66 (16.7%)	0.59
Progressive	42/64 (65.6%)	50/66 (75.8%)	0.20
Symptoms reported by patient - N (%)			
Paresthesia/Dysesthesia	52/65 (80.0%)	48/64 (75.0%)	0.49
Pain	35/65 (53.8%)	34/64 (53.1%)	0.93
Ataxia	34/65 (52.3%)	30/64 (46.9%)	0.53
Topography of reported symptoms - N (%)			
Purely LL	27/65 (41.5%)	29/65 (44.6%)	0.72
Including UL	33/65 (50.8%)	28/65 (43.1%)	0.38
Asymmetry	23/60 (38.3%)	20/63 (31.7%)	0.44
Clinical characteristics at the first clinical exam			
Delay - median year (25^{th}-75^{th} percentile)	2.9 (1.0-6.0)	3.9 (1.6-7.1)	0.20
SNN Score - median (25^{th}-75^{th} percentile)	9.9 (5.1-11.0)	9.8 (5.1-11.0)	0.73
Symptoms reported by patient - N (%)			
Paresthesia	51/65 (78.5%)	47/65 (72.3%)	0.41
Dysesthesia	34/65 (52.3%)	37/65 (56.9%)	0.59
Pain	36/65 (55.4%)	32/65 (49.2%)	0.48
Topography of reported symptoms - N (%)			
Purely LL	18/65 (27.7%)	13/65 (20.0%)	0.30
Including UL	46/65 (70.8%)	50/65 (76.9%)	0.42
Asymmetry	25/65 (38.5%)	22/64 (34.4%)	0.63
Clinical signs at clinical exam - N (%)			
Purely PTS altered (unmyelinated sensory fibers)	9/63 (14.3%)	3/59 (5.1%)	0.08
Purely DSS altered (myelinated sensory fibers)	14/63 (22.2%)	15/59 (25.4%)	0.67
PTS + DSS altered	38/63 (60.3%)	38/59 (64.4%)	0.64
Face included	8/61 (13.1%)	11/60 (18.3%)	0.43
Trunk included	9/62 (14.5%)	13/60 (21.7%)	0.30
Ataxia UL	25/65 (38.5%)	26/61 (42.6%)	0.63
Ataxia LL	39/65 (60.0%)	35/61 (57.4%)	0.76
Global areflexia	18/65 (27.7%)	22/63 (34.9%)	0.37
Dysautonomia	11/65 (16.9%)	15/65 (23.1%)	0.38
M-Rankin – median (25^{th}-75^{th} percentile)	2.0 (1.0-2.0)	2.0 (1.0-3.0)	0.54
Clinical characteristics at the last clinical exam			
Delay - median year (25^{th}-75^{th} percentile)	5.9 (3.9-11.3)	6.7 (3.4-12.2)	0.86
Symptoms reported by patient - N (%)			
Paresthesia	42/53 (79.2%)	33/50 (66.0%)	0.13
Dysesthesia	28/53 (52.8%)	21/50 (42.0%)	0.27
Pain	29/52 (55.8%)	24/49 (49.0%)	0.49
Topography of reported symptoms - N (%)			
Purely LL	14/53 (26.4%)	7/50 (14.0%)	0.11
Including UL	39/53 (73.6%)	42/50 (84.0%)	0.19

Asymmetry	21/53 (39.6%)	19/50 (38.0%)	0.86
Clinical signs at clinical exam - N (%)			
Purely PTS altered (unmyelinated sensory fibers)	5/51 (9.8%)	9/49 (18.4%)	0.21
Purely DSS altered (myelinated sensory fibers)	13/51 (25.5%)	14/49 (28.6%)	0.72
PTS + DSS altered	29/51 (56.9%)	34/49 (69.4%)	0.19
Face included	8/51 (15.7%)	6/50 (12.0%)	0.59
Trunk included	4/51 (7.8%)	12/50 (24.0%)	***0.02***
Ataxia UL	22/53 (41.5%)	26/49 (53.1%)	0.24
Ataxia LL	38/53 (71.7%)	32/49 (65.3%)	0.48
Global areflexia	21/53 (39.6%)	24/50 (48.0%)	0.39
Dysautonomia	15/53 (28.3%)	15/50 (30.0%)	0.85
Rankin – median (25th-75th percentile)	2.0 (1.0-3.0)	2.0 (1.0-3.0)	0.91
Associated autoimmune diseases - N (%)	22/65 (33.8%)	20/66 (30.3%)	0.66
SS	10/64 (15.6%)	9/64 (14.1%)	0.80
SLE	3/64 (4.7%)	2/64 (3.1%)	0.64
AIH	2/64 (3.1%)	2/64 (3.1%)	1.00
Inflammatory pathology of CNS	1/64 (1.6%)	3/64 (4.7%)	0.31
CIBD	1/64 (1.6%)	0/64 (0.0%)	0.31
Sarcoidosis	1/64 (1.6%)	1/65 (1.5%)	0.99
Other	4/64 (6.2%)	3/64 (4.7%)	0.70
Other associated diseases - N (%)			
Cancer	3/64 (5.0%)	5/64 (7.8%)	0.38
Biological data - N patient with abnormal result (%)			
CRP	4/60 (6.7%)	5/58 (8.6%)	0.69
Glycemia	4/59 (6.8%)	2/63 (3.2%)	0.35
HbA1c	3/48 (6.3%)	1/45 (2.2%)	0.34
Creatinine	1/61 (1.6%)	1/63 (1.6%)	0.98
SGPT	2/62 (3.2%)	6/63 (9.5%)	0.08
SGOT	2/62 (3.2%)	7/63 (11.1%)	0.15
GGT	5/61 (8.2%)	6/63 (9.5%)	0.79
ALP	1/60 (1.7%)	3/62 (4.8%)	0.32
TSH	0/54 (0%)	2/57 (3.5%)	0.16
ANA	25/60 (41.7%)	18/63 (28.6%)	0.12
Anti-dsDNA antibodies	2/43 (4.7%)	2/54 (3.7%)	0.81
Anti-SSA/SSB antibodies	8/49 (16.3%)	6/59 (10.2%)	0.34
Anti-mitochondrial antibodies	0/28 (0%)	0/24 (0%)	0.57
ANCA	2/39 (5.1%)	0/29 (0%)	0.21
Rheumatoid factor	5/39 (12.8%)	4/40 (10%)	0.69
Cryoglobulins	2/38 (5.3%)	3/40 (7.5%)	0.68
Complement	2/42 (4.8%)	3/38 (7.9%)	0.56
Proteinorachia	7/40 (17.5%)	8/40 (20.0%)	0.77
Treatment - N (%)	35/64 (54.7%)	30/65 (46.2%)	0.33
Corticosteroid	18/64 (28.1%)	20/65 (30.8%)	0.74
IV immunoglobulins	18/64 (28.1%)	19/65 (29.2%)	0.89
Azathioprine	10/64 (15.6%)	9/65 (13.8%)	0.77
Cyclophosphamide	4/64 (6.3%)	3/65 (4.6%)	0.68
Rituximab	2/64 (3.1%)	1/65 (1.5%)	0.55
Mycophenolate/mofetil	1/64 (1.6%)	4/65 (6.2%)	0.17
Treatment effects - N (%)			
Improvement	8/35 (22.9%)	8/30 (26.7%)	0.72
Stabilization	17/35 (48.6%)	12/30 (40.0%)	0.49
Failure	7/35 (20.0%)	5/30 (16.7%)	0.73
Unclear	2/35 (5.7%)	5/30 (16.7%)	0.15
Electrophysiological data (ENMG)			

Delay - median year (25^{th}-75^{th} percentile)	3.7 (1.8-8.2)	6.2 (2.1-10.5)	0.11
Sensory nerve conduction - mean N ± SD (mean %)			
Upper limbs)			
N tested nerves	5.0 ± 1.4	4.6 ± 1.5	0.14
N abnormal SNAPs	3.9 ± 2.2 (77.1%)	3.5 ± 2.4 (70.5%)	0.34
N abolished SNAPs	1.5 ± 1.9 (30.0%)	1.4 ± 1.8 (28.4%)	0.78
N abnormal SNAPs <50% LLN	1.6 ± 1.7 (30.3%)	1.6 ± 2.0 (33.6%)	0.75
N abnormal SNAPs >50% LLN	0.9 ± 1.3 (16.9%)	0.4 ± 1.0 (8.5%)	***0.01***
Lower limbs			
N tested nerves	3.4 ± 0.9	3.2 ± 1.0	0.21
N abnormal SNAPs	2.6 ± 1.5 (76.6%)	2.1 ± 1.5 (70.6%)	0.07
N abolished SNAPs	1.6 ± 1.6 (49.2%)	1.4 ± 1.5 (47.3%)	0.42
Motor nerve conduction (all limbs) - mean N ± SD (mean %)			
N tested nerves	5.8 ± 2.2	6.0 ± 1.8	0.93
N nerves with normal pattern	4.7 ± 2.0 (74.5%)	4.9 ± 2.1 (80.8%)	0.81
N nerves with axonal pattern	0.6 ± 1.0 (8.4%)	0.4 ± 0.6 (6.6%)	0.88
N nerve with primary demyelinating pattern	0.2 ± 0.9 (3.6%)	0.1 ± 0.8 (1.8%)	0.06
N nerve with mixed axonal and demyelinating pattern	0.9 ± 1.3 (13.5%)	0.6 ± 1.0 (10.9%)	0.31

AIH: autoimmune hepatitis; ALP, alkaline phosphatise; ANA: anti-nuclear antibody; ANCA, antineutrophil cytoplasmic antibodies; anti-dsDNA, anti-double stranded DNA antibodies; CIBD: chronic inflammatory bowel disease; CNS: central nervous system; CRP, C-reactive protein; DSS: Deep and superficial sensation; ENMG, electroneuromyography; F: female; GGT, gamma-glutamyl transferase; HbA1c, glycosylated hemoglobin A1c; IBD, inflammatory bowel disease; IV: intra-venous; LL, lower limbs; LLN, lower limit of the normal; M: male; M-Rankin, modified-Rankin; N: number; OSN, other sensory neuropathies; PTS: pain and temperature sensation; SFN, small fiber neuropathy; SGOT: serum glutamooxaloacetate transferase; SGPT: serum glutamopyruvate transferase; SLE: Systemic lupus erythematous; SNAPs, sensory nerve action potentials; SNN: sensory neuronopathy; SS: Sjögren syndrome; SSA, Sjögren syndrome A; SSB Sjögren syndrome B; TSH, thyroid-stimulating hormone; UL, upper limbs. The p-values in bold and italic with the symbol "*" are statistically significant after Bonferroni-correction. The p-values in bold and italic without the symbol "*" are ≤0.05 but not statistically significant after Bonferroni-correction.

Supplementary Figure S1 Identification of IgG subclasses of anti-FGFR3 antibodies and temporal evolution of anti-FGFR3 antibody concentrations.
IgG1 (A), IgG2 (B), IgG3 (C), IgG4 (D) signals expressed as z-score for anti-FGFR3 antibody-positive and negative patients obtained by indirect ELISA. Positivity was defined as $z \geq 3$ compared to anti-FGFR3-negative patients (dotted line). Twenty anti-FGFR3-positive sera were tested and antibodies were IgG1 in 14, IgG3 in 3 and IgG4 in 6 patients. IgG2 were not found. (E) Frequency of the different patterns of IgG subclasses found in the anti-FGFR3-positive patients. Anti-FGFR3 antibodies were only IgG1 in 8 patients, only IgG4 in 3 while IgG3 antibodies were always associated with IgG1. Of the 6 patients with IgG4 antibodies, 5 were women, 5 had SNN and 1 SFN, 3 had an autoimmune context. The IgG4 patients did not differ from the 14 patients without IgG4 antibodies in terms of sex, age, type of neuropathy, presence of an immune context, M-Rankin score, and response to treatment. (F) Comparison of anti-FGFR3 antibody concentrations in a first sample with those in a later sample ("second sample") that we analyzed for 38 patients (33 anti-FGFR3-negative and 5 anti-FGFR3-positive patients). The dotted line represents the threshold of positivity. The red lines show the temporal evolution of anti-FGFR3 antibody concentrations in positive patients and the black lines those in negative patients. The Wilcoxon test was used to compare the anti-FGFR3 antibody concentrations between the two times of sampling. The median delay between the both samples was 6.3 (4.5-15.0) months. All the second samples were interpreted as the first (figure 3F) and the antibody concentrations in the second sample of the anti-FGFR3-positive patients were not significantly different from those of the first sample ($P = 0.19$).


A
Z-score for anti-IgG1 ELISA
z=3
Anti-FGFR3-negative patients
Anti-FGFR3-positive patients
B
Z-score for anti-IgG2 ELISA
z=3
Anti-FGFR3-negative patients
Anti-FGFR3-positive patients
C
Z-score for anti-IgG3 ELISA
z=3
Anti-FGFR3-negative patients
Anti-FGFR3-positive patients
D
Z-score for anti-IgG4 ELISA
z=3
Anti-FGFR3-negative patients
Anti-FGFR3-positive patients
E
Frequency (%)
Only IgG1
Only IgG4
IgG1 and 4
IgG1 and 3
IgG1, 3 and 4
F
Concentration of anti-FGFR3 antibodies (μg/mL)
Anti-FGFR3-negative patients
Anti-FGFR3-positive patients
P = 0.19
P = 0.03
First sample
Second sample