



## Prevalence of antibodies to ganglioside and Hep 2 in Gaucher, Niemann – Pick type C and Sanfilippo diseases



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### ABSTRACT

Lysosomal Storage Diseases (LSDs) are rare genetic diseases, the majority of which are caused by specific lysosomal enzyme deficiencies and all are characterized by malfunctioning lysosomes. Lysosomes are key regulators of many different cellular processes and are vital for the function of the immune system. Several studies have shown the coexistence of LSDs and immune abnormalities. In this study, we investigated the presence of autoantibodies in the plasma of patients with Gaucher disease (GD;  $n = 6$ ), Sanfilippo Syndrome B (SFB;  $n = 8$ ) and Niemann – Pick type C disease (NPC;  $n = 5$ ) before and following Miglustat treatment ( $n = 3$ ). All were examined for antibodies to antigens of Hep-2 cells and antiganglioside antibodies (AGSA). No autoantibodies were detected in GD patients. 3/8 SFB patients showed only AGSA (2/3 IgM / IgG; 1/3 IgG), 3/8 only anti-Sm E/F and 2/8 showed both IgM / IgG or IgG AGSA and anti-Sm E/F. 3/5 NPC patients showed AGSA (2/3 IgM and IgG, 1/3 IgM) and one anti-Sm E/F and IgM AGSA. Following treatment one patient with no AGSA developed IgM AGSA and two with both IgG and IgM showed only IgG AGSA. In our study, investigating similar numbers of patients, autoantibodies were observed in NPC and SFB patients but not in GD patients. Our findings suggest that, independently of the development of an autoimmune disease in patients with LSDs, there seems to be an autoimmune activation that differs in different disorders. Further studies including more patients, also at different stages of disease and treatment, are needed in order to get further insight into the immune irregularities associated with different LSDs and their significance.

### 1. Introduction

Lysosomal Storage Diseases (LSDs) are a group of > 70 different rare genetic diseases which can be the result of defects in lysosomal enzymes, lysosomal membrane proteins, proteins involved in the posttranslational modification, transport and delivery of lysosomal enzymes to lysosomes, activator proteins that are essential for the *in vivo* activity of lysosomal enzymes as well as non-enzymatic soluble lysosomal proteins [1,2]. Irrespective of the primary cause all LSDs are characterized by the malfunctioning of lysosomes. Over the years, lysosomes have emerged as key regulators of many different cellular processes including signaling and regulation of metabolism. Their dysfunction thus, leads not only to primary lysosomal dysfunction but

also to the perturbation of many different cellular pathways generating a cascade of events that are believed to underlie the pathology of LSDs [3,4].

Lysosomes are vital components of immune cell processes and several studies, both in animal models and patients, have shown the coexistence of LSDs and immune irregularities and the dysfunction of the immune system has been implicated in the pathogenetic process in many LSDs [5–13].

In the present study we investigated the presence of autoantibodies to Hep-2 cells and AGSA in the plasma of patients with Gaucher disease (GD; OMIM ID: 230800, 230900, 231000), Sanfilippo Syndrome B (SFB; OMIM ID: 252920) and Niemann – Pick type C (NPC; OMIM ID: 257220, 607625) disease.

**Abbreviations:** AGSA, Antiganglioside antibodies; GD, Gaucher disease; LSDs, Lysosomal storage diseases; NPC, Niemann Pick type C disease; SFB, Sanfilippo B syndrome; PM-Scl-70, Polymyositis - Scleroderma-70; Scl-70, Scleroderma-70; Ku:Ku antigen(p70/p80)CENP A,B,C, Centromere proteins A,B,C; AMA-M2, anti-mitochondrial antibodies to M2 antigen; RNP, ribonucleoprotein; SS-A, Sjögren's antigen A; SS-B, Sjögren's syndrome antigen B; Jo-1, Histidyl-tRNA synthetase antigen; rib-P-Protein, Ribosomal P protein; Sm, Smith antigen (B,B',D,E,F,G proteins)

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**Table 1**  
Immunological findings in patients with Gaucher and Sanfilippo B disease.

Disease	Patients	Age	Anti-Ganglioside Antibodies Immunoglobulin G Immunoglobulin M (IgG) (IgM)	Antibodies to Sm-E/F Antibodies Immunoglobulin G (IgG)
GD1	1	55 years	- -	-
	2	67 years	- -	-
GD2	3	1 month	- -	-
	4	2 months	- -	-
GD3	5	13 months	- -	-
	6	17 years	- -	-
SFB	1	11 months	GQ1b + +, GT1b + +, GD1a + GD1b + +, GD1a + + + GM3 + +, GM2, GM1 + +	+ w
	2	3 years	GQ1b + +, GT1b +, GD1b + +, GD1a + GD1a + + +, GM3 + +, GM2 + +, GM1 + + +	-
	3	4 years	GT1b +, GD1b + +, GD1a + +, GD1b +, GD1a + GM3 +, GM2 + +, GM1 +	-
	4	5 years	-	+
	5	6 years	GT1b +, GD1b +, GD1a + -	-
	6	10 years	- GQ1b +	+
	7	13.5 years	- -	+
	8	18 years	- -	+

Abbreviations: Antibodies to Sm-E/F antibodies: + w: weekly positive, +: positive. Antiganglioside Antibodies signal intensity (EUOLINESCAN Flatbed scanner) +: 11–25; + +: 26–50, + + + > 50. GD1: Gaucher disease type 1, GD2: Gaucher disease type 2, GD3: Gaucher disease type 3.

## 2. Patients and methods

### 2.1. Patients

A total of 19 patients were studied. They included 6 patients with GD, 5 with NPC and 8 with SFB disease.

The GD group included: type 1 (GD1), type 2 (GD2) and type 3 (GD3) patients.

All the SFB group patients had the severe form of the disease.

All samples from GD and SFB patients were obtained on diagnosis and prior to the initiation of any treatment (Table 1).

The NPC group included patients with severe neonatal disease (NPC/1, NPC/2) and patients with later onset progressive disease (NPC/3, NPC/4, NPC/5). Samples from all NPC patients were available before the initiation of treatment. In 3 patients samples were also available at different time points after the initiation of Miglustat treatment (Table 2).

The study was conducted according to the guidelines of the Ethics Committee of the Institute of Child Health.

### 2.2. Methods

Plasma samples from all patients were examined for autoantibodies to gangliosides (GM<sub>1</sub>, GM<sub>2</sub>, GM<sub>3</sub>, GD<sub>1a</sub>, GD<sub>1b</sub>, GT<sub>1b</sub>, GQ<sub>1b</sub>), using the qualitative Euroline immunoblotting assay. Visual evaluation of test strips was initially performed according to the instructions of the manufacturer. The strips were then scanned and evaluated with EuroLineScan Flatbed scanner (Euroimmune, Germany).

Autoantibodies to antigens of Hep-2 cells (PM-Scl-70, Scl-70, Ku,

CENPA-C, AMA-M2, RNP, SS-A (60 kDa), SS-A (52 kDa), SS-B, CENP B, Jo-1, rib-P-Protein, Sm) were also detected using the qualitative Euroline-WB assay. The assay was carried out according to the manufacturer's protocol. In particular the provided evaluation matrix with control strip was used for visually evaluating the signals of the bands of the tested samples (Euroimmune, Germany).

## 3. Results

In the GD group of patients, no autoantibodies to the antigens studied were detected.

In the SFB group autoantibodies were detected in the majority of patients. 3/8 SFB patients showed only AGSA (2/3 IgM / IgG; 1/3 IgG), 3/8 only anti-Sm E/F and 2/8 showed both IgM / IgG or IgG AGSA and anti-Sm E/F (Table 1).

In the NPC group AGSA were detected in all but one treatment naïve patients. In particular 3/5 NPC patients showed AGSA (2/3 IgM and IgG; 1/3 IgM) and one IgM AGSA and anti-Sm E/F. In 3/5 patients, samples were also available at different time points following the initiation of treatment (Miglustat). Although on diagnosis, no AGSA were detected in NPC/1 patient, a response, involving IgM only AGSA, was detected following treatment. In both NPC/2 and NPC/5 patients, following treatment, only IgG AGSA were detected whereas both IgG and IgM antibodies were detected prior to treatment (Table 2). Patients from all groups studied had normal immunoglobulin levels (results not shown).

## 4. Discussion

Autoantibody responses both to storage material and molecules that are not themselves stored are in general considered uncommon in LSDs. Furthermore although a possible role of autoantibodies in the pathophysiology of GM2 gangliosidosis has been suggested [13] overall their role and contribution to disease pathologies remains unknown and merits further studies.

In GD, the accumulation of undigested glucocerebroside in macrophages as well as the accumulation of lyso-glucosylceramide are associated with a chronic stimulation of the immune system, which is believed to contribute to the pathology of the disorder including the increased risk for myeloma observed in GD patients [7–9,14]. In particular, the presence of increased levels of autoantibodies in the sera of GD type 1 patients has been shown in two studies. In the first, Shoenfeld et al. [15] investigated 43 patients for the presence of autoantibodies against 14 autoantigens and found autoantibodies in 33/43 of the patients. The increase in the incidence of autoantibodies tested ranged from 11% for anti-RNP, pyruvate dehydrogenase and anti-DNA antibodies to 57% for rheumatoid factor. They were considered as natural non-pathogenic autoantibodies. In the second more recent study, Seratrice et al. [16] studied 40 GD type I patients, 15 of whom were splenectomized whereas 37/40 of them were under treatment. They were able to detect autoantibodies, such as antinuclear, anticardiolipin, antiphospholipid, and anti-ganglioside antibodies in 52% of the patients compared to 26% of the control group. Antiphospholipid antibodies were the most frequently detected antibodies (30% in the patients vs 5% in the control group). On the other hand, antiganglioside antibodies were detected in 5/40 patients (12% in the patients vs 15% in the control group). Again, there was no clear association of the detected autoantibodies to clinical manifestation of autoimmune disease.

In our study, we did not detect any of the autoantibodies investigated in the untreated, non-splenectomized GD patients studied. This may be the result of the small number of patient and/or the age of the patients studied. However, our results indicate that there is no overt difference regarding autoantibody response between the three types of GD.

Immune dysfunction has been described and has been associated with clinical features of the disease in Niemann Pick C disease [11]. To

**Table 2**  
Immunological Findings in patients with Niemann-Pick type C patients with and without treatment.

Disease	Patients	Age	Treatment Period	Anti-Ganglioside Antibodies	
				Immunoglobulin G	Immunoglobulin M (IgM)
NPC	1	5 months	No	-	-
	1a	6 months	1 month	-	GT1b +
	1b	13 months	8 months	-	GD1a +
	1c	17 months	12 months	-	GT1b (+)
	2	6 months	No	GT1b+, GD1b+, GD1a +	GT1b+, GD1a+
	2a	1 year	7 months	GM3+, GM2+, GM1 +	GD1a + +
				-	GD1b + +, GT1b +,
				GM3 +	GM2 + +, GM1 + +
	3	2 years	No	-	GT1b + +
	4	6 years	No	-	GT1b +
	5	12 years	No	GT1b+, GD1b + +, GD1a+, GD1a +	GM3 +, GM2 +, GM1 + +
	5a	13 years and 9 months	1 year and 7 months	GM1 + +, GM2 +, GM3 + -	GT1b +, GD1a, GD1b (+)
	5b	14 years and 7 months	2 year and 5 months	GM1 +, GD1a +, GD1b + -	-

Abbreviations: Antibodies to Sm-E/F antibodies +: positive. Antiganglioside Antibodies signal intensity (EUROLINESCAN Flatbed scanner): (+) 6–10; +: 11–25; ++: 26–50, +++ > 50. NPC Niemann-Pick type C disease.

our knowledge there have been no published results regarding the study of autoantibodies in the disease.

NPC is a neurodegenerative LSD characterized by the accumulation of unesterified cholesterol and various sphingolipids in the lysosomes and late endosome. Among the latter, GM2 and GM3 are well characterized storage components of NPC [17]. Reduction in the levels of gangliosides, either through inhibition of GM3 synthase or the inhibition of glucosylceramide synthase by N-butyldeoxynoirmycin (Miglustat), has been shown to result in the attenuation of the neuropathology of NPC and the protection of PC12 neuronal rat cells [18–20]. In fact, Miglustat is now an approved drug for the treatment of NPC leading to stable or improved neurological manifestations in the majority of patients studied [21].

In our study, IgM and IgG autoantibodies to gangliosides were detected in 4/5 untreated patients. Following treatment with Miglustat, this autoimmune response persisted in patients NPC/2 and NPC/5 but only IgG antibodies were detected. On the other hand, in patient NPC/1 a weak reactivity involving IgM AGSA was observed. It is difficult to draw any conclusions from this small cohort of patients regarding a possible correlation between AGSA, response to treatment and disease progression. However, it is worth noting that patient NPC/1 who had no AGSA prior to treatment and a rather limited positivity after its initiation, apart from slight clumsiness and minor impairment in the motor coordination at all developmental stages, remains free of neurological manifestations at the age of 11 years. Patient NPC/5, already showing neurological deficit when treatment was initiated, showed a stabilization of her clinical picture, whereas patient NPC/2 showing an early onset severe disease died approximately one year after the initiation of treatment [22,23]. SFB syndrome or mucopolysaccharidosis IIIB, is a disorder with severe neurological manifestations characterized by the accumulation of partially degraded heparan sulphate oligosaccharides in tissues as well as the secondary storage of gangliosides and cholesterol [24]. Studies investigating the role of immune response in the neuropathology of the disease using SFB mice, have shown significant upregulation of immune related genes, involving a broad range of immune cells and molecules. Furthermore, IgG autoantibodies to brain protein were identified in the sera of SFB B mice [6].

As in NPC, where secondary accumulation of gangliosides also occurs, AGSA were detected in SFB patients. Furthermore 5/8 patients showed anti-Sm E/F antibodies, which are highly specific for Systemic Lupus Erythematosus [25]. Nevertheless none of our patients had clinical manifestations of autoimmune disease.

Gangliosides are a subgroup of glycosphingolipids characterized by the presence of sialic acid residues. They are found in plasma membrane lipid domains where they are involved in the modulation of diverse cellular functions. They are especially abundant in the brain and they play an important role in its development and maintenance [26–28]. Autoantibodies against gangliosides constitute an important component of several subtypes of autoimmune neuropathies. Anti-GM1 and anti-b series ganglioside IgM antibodies have been associated with various chronic forms of autoimmune neuropathies such as multifocal motor neuropathy and chronic ataxic neuropathy [29–31]. On the other hand, IgG antibodies have been associated with various acute forms of autoimmune neuropathies, including acute motor axonal neuropathy, acute motor-sensory axonal neuropathy and sensory ataxic neuropathy or ataxic Guillain-Barre syndrome [32–34].

In our study, both IgG and IgM class AGSA, either alone or in co-existence, were detected in NPC and SFB patients. Furthermore, IgG class antibodies persisted in the treated NPC patients who prior to treatment had both IgG and IgM antibodies, whereas a weak reactivity involving IgM AGSA was observed in a patient who had no AGSA prior to treatment. The limited number of patients studied makes it difficult to draw any conclusions regarding the significance of these findings.

In conclusion then, we were able to identify a broader spectrum of immunological findings in patients with SFB and NPC disease compared to GD patients. Our findings suggest that, independently to the development of an autoimmune disease, in patients with LSDs there seems to be an activation of autoimmune response towards specific antigens, including secondary stored molecules, that differ in different disorders. Clearly further studies, including more patients also at different stages of disease, are required in order to get further insight into the immune irregularities associated with different LSDs and their implication.

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## Conflict of Interests

H Michelakakis has received honoraria and travel reimbursements from Genzyme-Sanofi, Actelion, research grant from Actelion. E. Dimitriou has received travel reimbursements from Genzyme-Sanofi and Shire.

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