THE ROLE OF GLUCOCEREBROSIDASE IN NEURODEGENERATION ASSOCIATED TO GAUCHER'S DISEASE AND OTHER NEURODEGENERATIVE DISEASES

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Background

Mutations in the GBA gene increase the risk of Parkinson's Disease (PD). GBA variants predict a more rapid progression of cognitive dysfunction in parkinsonian patients. However, the precise frequency of GBA mutations in other dementias and neurodegenerative disorders is still unclear. Moreover, whether the glucocerebrosidase enxyme (GCase) activity may represent a biomarker in PD is still matter of debate.

Objective

To describe the prevalence of GBA mutations in several neurodegenerative diseases and to assess the frequency of prodromal parkinsonism in Gaucher's Disease (GD) patients; to evaluate GCase activity in peripheral blood mononuclear cells (PBMCs), fibroblasts and human induced pluripotent stem cell (iPSC)-derived dopaminergic neurons (DaNs) in a cohort of Parkinson's Disease (PD) patients with and without GBA mutations.

Results

The frequency of GBA mutations in Parkinson Disease and Lewy Body Disease (LBD) patients was respectively 7,1% and 4.9%. Among LBD L444P mutation was significantly more prevalent. GBA mutations were not significantly increased in Amyotrophic Lateral Sclerosis (ALS) ad Alzheimer Disease (AD) (Fig. 1).
The neurological examination of GD patients (n=30) revealed a high frequency (80%) of non-motor symptoms, especially R.E.M. Behaviour Disorder (RBD), followed by constipation and depression. Motor symptoms were found in 14 GD patients (60%) (Fig. 2). Nine of them (30%) had at least a family member affected by PD and six of them(20%) had a possibile positive family history of parkinsonism.
PBMCs (Fig.3), fibroblasts (Fig.4) and DaNs (Fig.5) from GBA mutated PD showed a lower GCase activity compared to non-GBA PD and controls. GCase protein level was decreased in GBA DaNs (Fig.6). Treatment with ambroxol partially restored GCase activity and protein amount in GBA-mutated DaNs and fibroblasts to levels similar to controls.

Motor Symptoms (14/30; 60%)	Bradykinesia	6 (20%)
	Resting Tremor	3 (10%)
	Postural Tremor	2 (6%)
	Rigidity	2 (6%)
	Other (eye movement impairment, RLS, arm swinging reduction)	7 (23%)
Non Motor Symptoms (24/30; 80%)	RBD	10 (30%)
	Constipation	7 (23%)
	Depression	5 (16%)
	Urinary Urgency	1 (3%)
	Hyposmia	1 (3%)

Fig. 2 Neurological feautures in Gaucher Disease patients

Discussion

GBA mutations have an high frequency in PD and LBD, whereas any association was found between GBA mutations and ALS or AD. GD has a higher incidence of parkinsonism compared to general population, especially non-motor symptoms which could represent prodromal markers of PD in GD. The detection of a low GCase activity in patient-derived cells may represent a new possible biomarker for GBA-PD.

Conclusions

GBA mutations are the most common genetic risk factor for PD and LBD, whereas they seem not to play a role in others neurodegenerative diseases. Measurement of GCase could be a possible biomarker for GBA-PD and ambroxol may be a promising therapy for GBA-associated neurodegeneration.

Materials and Methods

Clinical evaluation of a cohort of GD patients and relatives and a selection of patients with neurodegenerative disorders. DNA was extracted from all patients and GBA mutation screening performed. PBMCs were isolated from fresh blood and fibroblasts obtained from skin biopsies. iPSCs were generated from viral reprogramming of fibroblasts and further differentiated into DaNs. GBA mutated fibroblasts and DaNs were treated with ambroxol. Chemiluminescent enzymatic assay and western blot analyses were performed.

	Mutations		Frequency	P value	OR (IC 95%)
PD	Tot: 10		7,1%	**0,005**	5,64 (1,52-20,87)
Tot. 140	*E326K*	*4*	*2,9%*	*0,15*	*3,25 (0,59-17,98)*
	N370S	*3*	*2,2%*		
	L444P	*3*	*2,1%*	***0,01***	*10,02 (1,19-84,10)*
LBD	Tot: 5		4,9%	0,07	3,78 (0,89-16,13)
Tot. 102	*E326K*	*2*	*2,0%*	*0,37*	*2,21 (0,30-15,91)*
	N370S	*0*	*0%*	*0,69*	*NA*
	L444P	*3*	*2,9%*	***0.03***	*NA*
ALS	Tot: 4		2,6%	0,30	1,96 (0,43-8,86)
Tot. 154	*E326K*	*2*	*1,3%*	*0,53*	*1,45 (0,20-10,43)*
	N370S	*2*	*1,3%*		
	L444P	*0*	*0%*	*0,36*	*2,92 (0,26-32,50)*
AD	Tot: 1		0,6%	0,41	0,42 (0,04-4,16)
Tot. 172	*E326K*	*0*	*0%*	*0,32*	*NA*
	N370S	*1*	*0,6%*		
	L444P	*0*	*0%*	*0,68*	*1,30 (0,08-20,91)*
CTR	Tot: 3		1,3%		
Tot. 223	*E326K*	*2*	*0,9%*		
	N370S	*1*	*0,4%*		
	L444P	*0*	*0%*		

Fig. 1. Results of GBA screening. PD: Parkinson's Disease; LBD: Lewy Body Dementia; ALS: Amyotrofic Lateral Sclerosis; AD: Alzheimer Disease; CTR: control. P value was calculated comparing each group of patients with controls. OR: odds ratio.

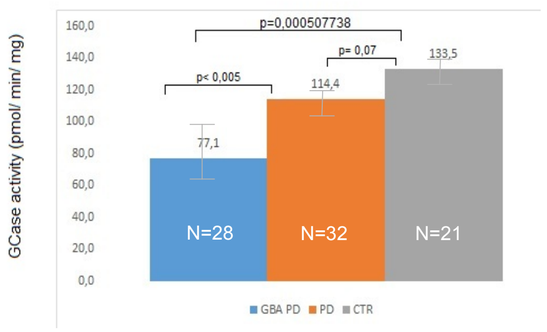


Fig.3 Gcase activity in PD-GBA vs PD and vs CTR

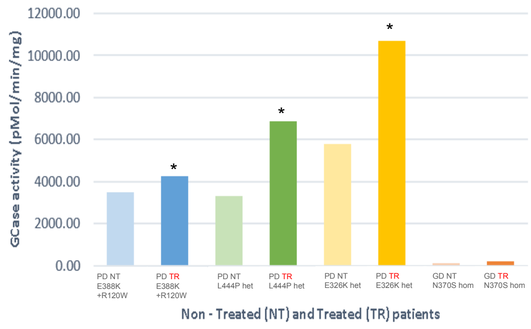


Fig.4 GCase activity in GBA mutated fibroblasts before and after Ambroxol treatment. Ambroxol improves glucocerebrosidase activity in fibroblasts with GBA heterozygous and homozygous mutations (*p < 0.05).

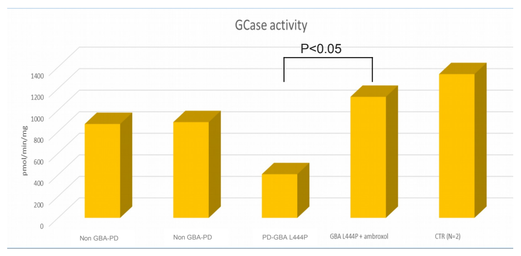
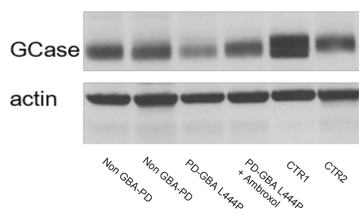


Fig. 5 GCase activity in DaNs from GBA mutated PD



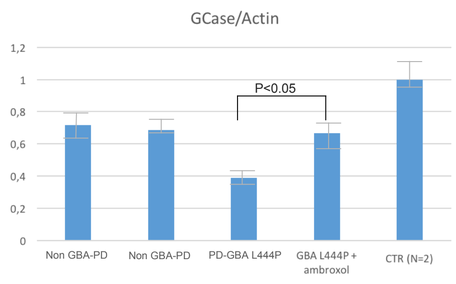


Fig.6 WB and densitometry of GCase in DaNs from non GBA-PD, GBA-PD with L444P het and CTR before and after ambroxol treatment.

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