Proteomic profiling of skin fibroblasts from patients with Parkinson's Disease carrying heterozygous variants of glucocerebrosidase and parkin genes

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Introduction

Pakinson's disease (PD) is a second most common neurodegenerative disorder of unknown pathogenic mechanisms. While the exact cause of PD is generally unknown, the development and progression of the disease is believed to be associated with environmental and genetic factors. Among them, the variants in *GBA* and *PARK2* genes have been reported to be important risk factors for developing PD. Glucocerebrosidase (GCase), also known as acid-β glucosidase (GBA) which is encoded by *GBA* gene, is involved in the hydrolysis of glucocerebrosides, an intermediate in glycolipid metabolism in cell membrane. Mutations of *GBA* gene have been classically associated with Gaucher's disease, a disorder involvement of the central nervous system. Growing evidence indicates that *GBA* heterozygous state may contribute as a genetic risk factor involved in the development of PD. On another hand, parkin, encoded by *PARK2* gene, is an E3 ubiquitin ligase and plays an important role in ubiquitination. It can tag proteins that are damaged or misfolded and its role also involves in damaged mitochondria and autophagy. *PARK2* mutations are a common cause of the early onset of PD. However, the mechanisms how these mutations cause PD remain largely unknown.

In this study, we investigate the protein expression patterns of skin fibroblasts from PD patients carrying heterozygous variants of *GBA* and *PARK2* genes in comparison to those of healthy controls. Fibroblasts of 2 PD patients carrying heterozygous *GBA* mutations and 2 PD patients carrying *PARK2* mutations, and 4 age-matched healthy controls were used. Mass spectrometry and label-free quantitative proteomics were performed. Proteins of interest were validated by western blot analysis. In addition, protein-protein interactions (PPIs) of those altered proteins was determined by STRING analysis.

Methods

Skin fibroblasts → Harvested at 80% confluence → Scraped, lyzed in lysis buffer, sonicated and centrifuged to obtain the soluble proteins

- In-solution tryptic digestion → Nano LC-MS/MS and Mascot search → Progenesis QI software
- One dimensional gel electrophoresis and Western blot → Chemiluminescent detection → Image Quant software

PPIs analysis

Table 1. Phenotypic and Genotypic data of Parkinson's disease patients and controls

	Name	Gender	Age (years)	Causative gene	Mutation (DNA)
Patients	PD-GBA-1	57	F	*GBA*	c.1309delG (V398fsX404) heterozygous
	PD-GBA-2	49	F	*GBA*	c.IVS2+1G>A heterozygous
	PD-PARK2-1	43	F	*PARK2*	c.2T>C
	PD-PARK2-2	41	F	*PARK2*	Exon 8 deletion
Controls	Normal-1	45	F	None	-
	Normal-2	53	M	None	-
	Normal-3	46	F	None	-
	Normal-4	45	F	None	-

Results

Table II List of the top 25 proteins with differentially altered expression levels from skin fibroblasts of patients with Parkinson's disease carrying heterozygous *GBA* and *PARK2* variants.

A. Proteins from skin fibroblasts of patients with PD carrying heterozygous GBA variants

#	Uniprot	Accession (Gene code)	Description	Peptide counts	Protein Scores	Fold changes ±SD	Best peptide Anova
1	P07355	ANXA2	Annexin A2	26	1700.34	1.97±0.44	$1.72x10^{-7}$
2	P62805	HIST1H4F	Histone H4	6	431.19	1.88±0.73	$8.28x10^{-10}$
3	P31949	S100A11	Protein S100-A11	6	254.60	1.63±0.76	$6.37x10^{-7}$
4	Q01995	TAGLN	Transgelin	16	850.10	1.62±0.46	$1.83x10^{-8}$
5	Q07020	RPL18	60S ribosomal protein L18	6	180.00	1.59±0.30	$1.72x10^{-7}$
6	P04908	HIST1H2AB	Histone H2A type 1-B/E	7	281.52	1.47±0.09	$1.48x10^{-7}$
7	P07437	TUBB	Tubulin beta chain	20	866.30	1.34±0.03	$8.28x10^{-6}$
8	Q96A08	HIST1H2BA	Histone H2B type 1-A	8	145.32	1.32±0.26	$2.46x10^{-6}$
9	P51991	HNRNPA3	Heterogeneous nuclear ribonucleoprotein A3	14	456.83	1.27±0.01	$1.22x10^{-4}$
10	P67809	YBX1	Nuclease-sensitive element-binding protein 1	11	252.44	1.27±0.37	$1.53x10^{-5}$
11	P02765	AHSG	Alpha-2-HS-glycoprotein	6	183.46	1.26±0.56	$1.47x10^{-5}$
12	P15880	RPS2	40S ribosomal protein S2	20	328.88	1.26±0.11	$1.22x10^{-3}$
13	P08670	VIM	Vimentin	46	2780.63	1.25±0.18	$2.79x10^{-8}$
14	O00159	MYO1C	Unconventional myosin-Ic	34	336.69	1.24±0.14	$4.32x10^{-3}$
15	P08865	RPSA	40S ribosomal protein SA	10	379.80	1.23±0.23	$2.72x10^{-2}$
16	Q9Y678	COPG1	Coatomer subunit γ-1	31	354.38	1.23±0.01	$3.14x10^{-4}$
17	Q15582	TGFBI	Transforming growth factor-β-induced protein ig-h3	20	546.28	1.21±0.22	$4.71x10^{-6}$
18	P23284	PPIB	Peptidyl-prolyl cis-trans isomerase B	15	550.47	1.20±0.23	$1.30x10^{-7}$
19	P07910	HNRNPC	Heterogeneous nuclear ribonucleoproteins C1/C2	18	522.89	1.19±0.00	$1.76x10^{-3}$
20	P40926	MDH2	Malate dehydrogenase, mitochondrial	17	546.50	1.19±0.04	$4.88x10^{-6}$
21	P09651	HNRNPA1	Heterogeneous nuclear ribonucleoprotein A1	14	500.11	1.19±0.06	$4.58x10^{-6}$
22	P12111	COL6A3	Collagen α-3(VI) chain	102	2434.47	1.18±0.01	$1.07x10^{-7}$
23	Q99715	COL12A1	Collagen alpha-1(XII) chain	82	1558.81	0.83±0.06	$3.19x10^{-4}$
24	P19105	MYL12A	Myosin regulatory light chain 12A	4	298.21	0.82±0.10	$4.88x10^{-3}$
25	P37802	TAGLN2	Transgelin-2	12	324.07	0.82±0.02	$1.48x10^{-4}$

B. Proteins differentially expressed in PD patients carrying heterozygous *PRKN2* mutations

#	Uniprot	Accession	Description	Peptide counts	Protein Scores	Fold changes ±SD	Best peptide Anova
1	P07437	TUBB	Tubulin β chain	16	476.78	1.77±0.16	$2.66x10^{-3}$
2	P60033	CD81	CD81 antigen	6	173.21	1.76±0.22	$1.09x10^{-2}$
3	P68104	EEF1A1	Elongation factor 1-α 1	15	520.37	1.75±0.51	$6.49x10^{-5}$
4	Q13813	SPTAN1	Spectrin α chain, non-erythrocytic 1	91	1606.84	1.65±0.56	$6.85x10^{-4}$
5	P04908	HIST1H2AB	Histone H2A type 1-B/E	4	245.77	1.64±0.52	$6.04x10^{-7}$
6	P00338	LDHA	L-lactate dehydrogenase A chain	15	739.16	1.58±0.41	$5.04x10^{-5}$
7	Q01082	SPTBN1	Spectrin beta chain, non-erythrocytic 1	71	1350.29	1.56±0.40	$2.89x10^{-3}$
8	P13804	ETFA	Electron transfer flavoprotein subunit alpha, mitochondrial	10	230.39	1.55±0.02	$2.88x10^{-3}$
9	O75369	FLNB	Filamin-B	89	1800.90	1.54±0.11	$7.31x10^{-6}$
10	Q6NZI2	PTRF	Polymerase I and transcript release factor	13	396.86	1.51±0.06	$6.01x10^{-3}$
11	P04075	ALDOA	Fructose-bisphosphate aldolase A	16	717.61	1.49±0.47	$4.81x10^{-5}$
12	P21589	NT5E	5'-nucleotidase	11	403.14	1.49±0.36	$1.73x10^{-3}$
13	Q9NZN4	EHD2	EH domain-containing protein 2	15	422.23	1.47±0.24	$1.04x10^{-2}$
14	P04406	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	16	709.19	1.46±0.25	$2.52x10^{-7}$
15	P32119	PRDX2	Peroxiredoxin-2	11	245.96	1.46±0.07	$7.45x10^{-4}$
16	P23634	ATP2B4	Plasma membrane calcium-transporting ATPase 4	37	678.12	1.45±0.47	$3.24x10^{-3}$
17	P15144	ANPEP	Aminopeptidase N	33	769.39	1.44±0.07	$4.44x10^{-3}$
18	P68363	TUBA1B	Tubulin α-1B chain	9	686.84	1.42±0.20	$3.43x10^{-5}$
19	P21291	CSRP1	Cysteine and glycine-rich protein 1	8	340.11	1.40±0.44	$2.42x10^{-4}$
20	Q13642	FHL1	Four and a half LIM domains protein 1	9	207.41	1.40±0.25	$9.79x10^{-5}$
21	P08133	ANXA6	Annexin A6	37	1269.01	1.38±0.03	$3.17x10^{-6}$
22	P08123	COL1A2	Collagen α-2(I) chain	34	1279.13	0.72±0.13	$1.06x10^{-5}$
23	P02751	FN1	Fibronectin	85	4542.86	0.72±0.02	$2.40x10^{-7}$
24	P07996	THBS1	Thrombospondin-1	36	1290.19	0.68±0.07	$5.24x10^{-5}$
25	P02452	COL1A1	Collagen α-1(I) chain	52	1901.37	0.65±0.18	$3.54x10^{-8}$

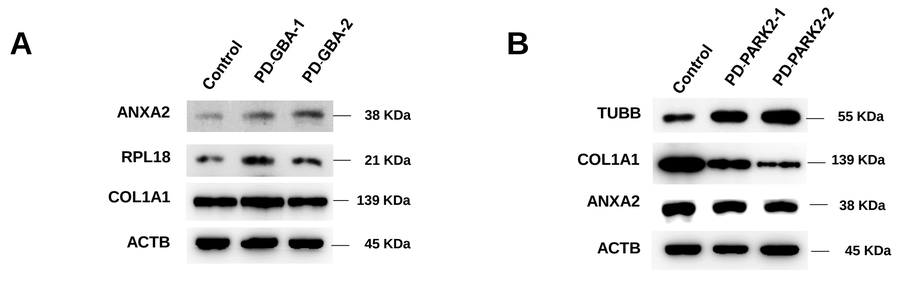

Figure 1. Validation of proteins expressed in pooled fibroblast samples of healthy controls, and in fibroblasts from patients with PD carrying *GBA* and *PARK2* mutations. (A) Western blots of ANXA2, RPL18 and COL1A1 in the GBA group. (B) Western blots of TUBB, COL1A1 and ANXA2 in the PARK2 group. ACTB was used for normalization of protein loading in each sample

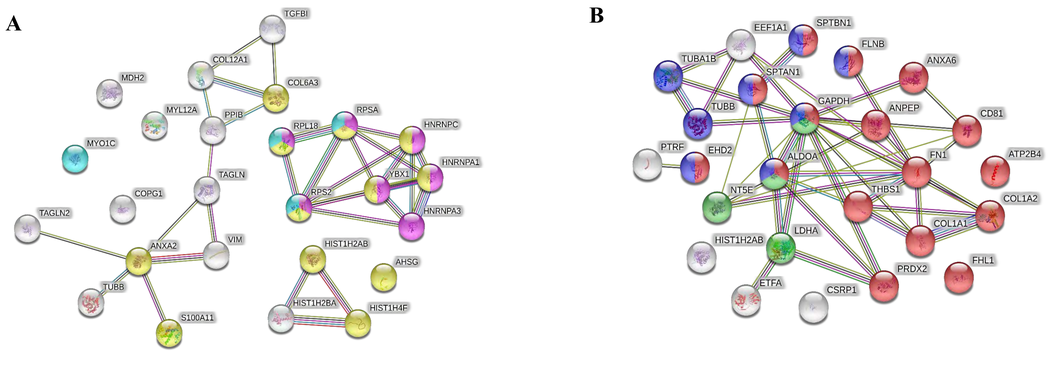

Figure 2. PPIs of the top 25 proteins differentially expressed in skin fibroblasts of patients with PD carrying (A) heterozygous GBA variants and (B) heterozygous parkin variants. PPI were mapped by STRING and categorized by biological processes and Gene Ontology. Yellow indicates proteins involved in negative regulation of macromolecule metabolic processes; purple indicates proteins involved in an mRNA metabolic process; cyan indicates proteins involved in targeting to the membrane; red indicates proteins involved in the regulation of biological quality; blue indicates proteins involved in cytoskeleton organization; and green indicates proteins involved in an NAD metabolic process.

Summary

- From LC-MS/MS and a label-free quantitative analysis, proteins were selected by expression changes of the top 25 proteins in similar direction (up or down) from all fibroblasts of *GBA* mutations or fibroblast of *parkin* mutations in comparison to healthy controls.
- The average expression of 5 and 11 proteins were altered >1.5 fold compared between those in the controls and the PD patients with *GBA* and *PARK2* variants, respectively (Table IIA and B). ANXA2 were most highly up-regulated in fibroblast carrying heterozygous *GBA* mutations, meanwhile the TUBB level is most highly upregulated in patient with heterozygous *PARK2* variants.
- The levels of some selected proteins including ANXA2, TUBB, RPL18, COL1A1 and ACTB in pooled fibroblast of healthy controls and each fibroblast' PD patient carrying *GBA* and *PARK2* were validated by immunoblotting and the results were similar to what we found from label-free MS quantitative analysis (Figure 1).
- Alteration of ANXA2 and TUBB levels in two variants may be involved in PD pathology; ANXA2 is reported to be involved in the autophagy regulation and Ca^{2+} homeostasis while TUBB is likely to be involved in the cytoskeleton organization.
- According to PPIs analysis, the GBA-PD patient fibroblasts displayed 12 proteins involved in negative regulation of macromolecule metabolic processes, 7 proteins involved in an mRNA metabolic process, and 4 proteins acted in targeting to the membrane.
- According to PPIs analysis, the PARK2-PD patient fibroblasts showed 16 proteins involved in the regulation of biological quality, 4 proteins acted in an NAD metabolic process and 8 proteins involved in cytoskeleton organization.

Acknowledgement

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