Preliminary characterization of lysosomal-related genes in two Tay Sachs variant B1 fibroblast cell lines

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Introduction

- Tay Sachs disease (**TSD**) variant B1 is a neurodegenerative lysosomal storage disease which, although rare, is the most frequent form of TSD in Northwestern Iberia;
- The mutation **p.R178H** (c.533G>A; rs28941770) associated with the TSD variant B1 leads to a mutant HexA protein with altered kinetics;
- **Human induced pluripotent stem cells** (**hiPSCs**) reprogrammed from patient cells are used as disease models preserving the patient's genetic background and being capable to undergo differentiation in almost all cell types (1);
- In the case of TSD variant B1 it is important to differentiate **hiPSCs** into **neuronal precursor cells** (**NPCs**) in order to contribute to the study of neuronal involvement in this rare disease;
- **Next Generation Sequencing** (**NGS**) can be used as a genomics checkpoint, in order to ensure the identity of the manipulated cells and establish their genetic profile.

Aim

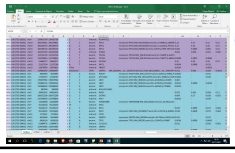
- The present work focus on the NGS characterization of lysosomal-related genes in two skin fibroblast cell lines from TSD variant B1 patients with the mutation p.R178H **prior** to the manipulations to generate hiPSCs and NPCs. Registering the gene variants at "time zero" will allow the eventual detection of future alterations.

(1) Anderson,R.H.,Francis,K.R. (2018) Modeling rare diseases with induced pluripotent stem cell technology. Mol Cell Probes Jan5. pii:S0890-8508(18)30001-X.

Material and Methods

TSD variant B1 patient fibroblast cell lines (L49 and L50)
(obtained from a cell bank)

gDNA extraction → **gDNA** → library preparation → NGS sequencing → Bioinformatic data analysis → NGS data analysis



SureCall — NGS data analysis software for non-bioinformaticians

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Results

Table 1 – Example of identified point variants in exons and their respective intronic flanking regions in the genes analyzed that were found in the respective fibroblast cell line.

Cell Line	Gene	Ref	Alt	ExonicFunc	AAChange	1000G_EUR	dbSNP	SIFT	PolyPhen	CADD
L49	LRP2	A	C	nonsynonymous SNV	NM_004525:exon64:c.T11996G:p.V3999G	0.018	rs79723119	tolerated	benign	likely benign
L49	MANBA	C	T	nonsynonymous SNV	NM_005908:exon17:c.G2482A:p.V828	0.024	rs75826658	**deleterious**	**possibly damaging**	likely benign
L49	MAN2A1	C	G	nonsynonymous SNV	NM_002372:exon13:c.C2020G:p.Q674E	.	rs150876984	tolerated	benign	likely benign
L49	PRKN	G	A	nonsynonymous SNV	NM_013988:exon8:c.C757T:p.R253C	0.0089	rs55830907	**deleterious**	benign	likely benign
L49	PEX1	G	A	nonsynonymous SNV	NM_000466:exon5:c.C899T:p.A300V	.	rs769114978	tolerated	benign	likely benign
L49	ABCA1	C	G	nonsynonymous SNV	NM_005502:exon24:c.G3516C:p.E1172D	0.031	rs33918808	tolerated	benign	likely benign
L49	**HEXA**	**C**	**T**	**nonsynonymous SNV**	**NM_000520:exon5:c.G533A:p.R178H**	**0.001**	**rs28941770**	**deleterious**	**probably damaging**	**likely deleterious**
L50	PARK7	G	A	nonsynonymous SNV	NM_001123377:exon5:c.G293A:p.R98Q	0.014	rs71653619	tolerated	benign	likely benign
L50	LRP2	T	C	nonsynonymous SNV	NM_004525:exon52:c.A10165G:p.I3389V	.	rs1402720085	**deleterious**	**probably damaging**	likely benign
L50	LRP2	C	T	nonsynonymous SNV	NM_004525:exon39:c.G7007A:p.R2336Q	.	rs147287428	tolerated	benign	likely benign
L50	LRP2	T	A	nonsynonymous SNV	NM_004525:exon37:c.A6256T:p.T2086S	.	rs146149181	**deleterious**	benign	likely benign
L50	LRRK2	C	G	nonsynonymous SNV	NM_198578:exon14:c.C1653G:p.N551K	0.064	rs7308720	**deleterious**	**probably damaging**	likely benign
L50	LRRK2	G	A	nonsynonymous SNV	NM_198578:exon30:c.G4193A:p.R1398H	0.066	rs7133914	tolerated	**possibly damaging**	likely benign
L50	HEXA	**C**	**T**	**nonsynonymous SNV**	**NM_000520:exon5:c.G533A:p.R178H**	**0.001**	**rs28941770**	**deleterious**	**probably damaging**	**likely deleterious**

Discussion and Conclusion

- Analyzing the NGS data we identified some variants of interest.
- Particularly relevant in further work with these cell lines may be the alterations in genes involved in other neurodegenerative diseases (such as PARK7, PRKN, LRRK2 or MANBA genes) and in genes coding proteins that involved in transport of molecules across cell membranes or organelle maintenance (such as LRP2, LRRK2, ABCA1, PEX1 and genes).

In the process of creating disease-specific cell models	Important	Carry out a broad genetic characterization of the different cell lines at all manipulation steps: from hiPSCs to NPCs
NGS data Differences among patient fibroblasts	Future	**NGS analysis** Differences in neuronal cell lines from these TSD variant B1 patients?

References:
(1) Anderson,R.H.,Francis,K.R. (2018) Modeling rare diseases with induced pluripotent stem cell technology. Mol Cell Probes Jan5. pii:S0890-8508(18)30001-X.