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Cerebellar cells derived from induced pluripotent stem cells in 3D culture generated from ARSACS patients as faithful disease model

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Objective 1. To generate *in vitro* disease model by creating ARSACS disease- specific cerebellar cells via iPSC from patients and healthy individuals as controls which will serve as a tool to depict disease-specific molecular markers. (1-10 months)

Patients.
Two ARSACS patients and 2 siblings were recruited through collaboration with Neurologists from Hospital 12 de Octubre, Madrid, Spain, or Hospital La Paz, Madrid, Spain. ARSACS patients previously genotyped for mutations in SACS gene (Sanchez et al., 2015) and their siblings were willing to collaborate in skin biopsy donation. Table 1 shows the patients genotypes, age and sex.

Individual	Gene	Allele 1	Allele 2	Phenotype	Age	Sex
1	SACS	wt	?	Healthy sibling of # 2		F
2	SACS	P3313QfsX11	R3792X	ARSACS	11	M
3	SACS	R276C	wt	Healthy sibling of # 4		F
4	SACS	R276C	P1302S	ARSACS	38	F

Skin biopsies. (Months 1-3) After signing Informed Consent and getting reprogramming permits according to Spanish legislation skin punch biopsies were obtained in collaboration with corresponding Departments of Neurology in each hospital. The primary fibroblast cell lines are generated from biopsies according to Raya et al., 2010. **Generation and characterization of iPSC**. (Month 4-6) Fibroblasts from patient (number 2 in table) and his sibling were reprogrammed using non-integrative Sendai virus (Cytotune, Life Technologies) technology. Currently we are selecting the pluripotent iPSC colonies according to their morphology and presence of pluripotency markers such as TRA-1-81. Three iPSC lines will be selected and characterized per patient. **Generation of 3D cerebellar cell culture from healthy iPS cells (**Months 1-6**).** The differentiation protocol to generate cerebellar cells, specifically Purkinje cells, is currently being optimized according to recently published study of Muruguma et al., 2015 who describe the generation of cerebellar 3D cell culture. For these purposes we are using iPSC line derived from healthy individual previously generated in our lab. The expression of cerebellar markers (Atoh1, Neph3, Calbindin, L7) throughout the differentiation protocol is being analyzed RT-PCR and immunohistolochemistry.