Neurogenetics
DOI 10.1007/s10048-015-0455-z

ORIGINAL ARTICLE

# Novel *SIL1* mutations cause cerebellar ataxia and atrophy in a French-Canadian family

**Anne Noreau [1,2] · Roberta La Piana [3,4] · Camille Marcoux [2] · FORGE Canada · Patrick A. Dion [1,2,5] · Bernard Brais [1,3,5] · Geneviève Bernard [6] · Guy A. Rouleau [1,5]**

Received: 2 June 2015 / Accepted: 24 July 2015


**Abstract** Two French-Canadian sibs with cerebellar ataxia and dysarthria were seen in our neurogenetics clinic. The older brother had global developmental delay and spastic paraplegia. Brain MRIs from these two affected individuals showed moderate to severe cerebellar atrophy. To identify the genetic basis for their disease, we conducted a whole exome sequencing (WES) investigation using genomic DNA prepared from the affected sibs and their healthy father. We identified two mutations in the *SIL1* gene, which is reported to cause Marinesco-Sjögren syndrome. This study emphasizes how the diagnosis of patients with ataxic gait and cerebellar atrophy may benefit from WES to identify the genetic cause of their condition.

**Keywords** Cerebellar · Ataxia · Whole exome sequencing · *SIL1* · Mutations

✉ Guy A. Rouleau
guy.rouleau@mcgill.ca

[1] Montreal Neurological Institute and Hospital, McGill University, 3801 University St., Room 636, Montreal, Quebec, Canada

[2] Department of Pathology and Cellular Biology, Faculty of Medicine, Université de Montréal, Montreal, Quebec, Canada

[3] Laboratory of Neurogenetics of Motion, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada

[4] Department of Neuroradiology, Montreal Neurological Institute and Hospital, McGill University, Montreal, Quebec, Canada

[5] Department of Neurology and Neurosurgery, McGill University, Montreal, Quebec, Canada

[6] Departments of Pediatrics, Neurology and Neurosurgery, Division of Pediatric Neurology, Montréal Children's Hospital, McGill University Heath Center, Montréal, Quebec, Canada

## Introduction

Hereditary ataxias are a complex group of disorders for which different modes of inheritance occur. The dominant forms, also known by spinocerebellar ataxias (SCA), are causes by mutations in 35 distinct genes [1]. The number of genes thus far reported to cause recessive forms has now reached 22. The best known recessive form is Friedreich ataxia (FA) which is caused by mutations in the *FRDA* gene. In the French-Canadian (FC) population, mutations in the *SACS* gene is a frequent cause of autosomal recessive ataxia, referred to as spastic ataxia of Charlevoix-Saguenay (ARSACS). Nonetheless, most cases of recessive ataxia in the FC population result neither from FA or ARSACS. Establishing the precise molecular diagnosis is essential in order to provide a precise diagnosis and offer genetic counseling. In addition, specific treatments can sometimes be offered [2, 3]. Among the recessive ataxias, Marinesco-Sjögren syndrome (MSS) (MIM#248800) is an infantile onset multisystem disorder that affects the brain, eyes, and skeletal muscles [4]. The presence of early onset cataracts and myopathy in association with cerebellar ataxia and atrophy characterizes MSS [4, 5]. Additional features can be found such as hypergonadotrophic hypogonadism, skeletal abnormalities, and short stature [5]. The locus was mapped to chromosome 5q31 by homozygosity mapping in two large consanguineous families, and the causative gene was found to be *SIL1* [5, 6]. *SIL1* mutations are found in a number of distinct populations such as Norwegian, Turkish, Finnish, Swedish, Italian, and Alsacian [6–8]. The triad—cerebellar atrophy, cataracts, and myopathy—was strongly associated with *SIL1* mutations [4][4].

Here, we report two siblings of FC descent affected by autosomal recessive cerebellar ataxia and atrophy caused by two novel mutations in the gene *SIL1*. The absence of cataracts and myopathy in this family suggests that *SIL1* mutations

Published online: 11 August 2015

should be considered in patients with cerebellar ataxia and atrophy, even when the classic clinical criteria of MSS are not fulfilled.

## Subjects

The proband (II.1) is the son of a non-consanguineous French-Canadian couple. His younger sister was also found to have signs of ataxia. Both their parents are unaffected, as are two half-siblings from the mother's side.

### Patient 1 (II.1)

The proband was seen by a pediatric neurologist at the age of 1 year for hypotonia and poor head control. The CT scan and MRI of the brain were performed and revealed a moderate to severe cerebellar atrophy, predominating at the vermis. The MRI also showed high T2 signal of the posterior periventricular white matter.

The patient was first evaluated by our neurogenetics team when he was 13 years old. The patient had been diagnosed early on with global developmental delay. He walked independently at the age of 7 years. No formal neuropsychological testing was performed. At that time, the clinical exam documented cerebellar ataxia and diffuse hypotonia and hyperreflexia.

He had difficulties at school, functioning at a grade 2 level when he reached the age of 17 years. Over time, the patient's hypotonia decreased, while lower limbs spasticity emerged. Physical examination at the age of 23 years revealed prominent dysarthria. Cranial nerve examination was significant for hypermetric saccades, abnormal smooth pursuits, and horizontal gaze-evoked nystagmus. The motor examination revealed spasticity of the lower more than the upper extremities, with brisk reflexes, sustained ankle clonus, and bilateral Babinski. The muscular power was conserved and no myopathic signs were evident at the neurological exam. Cerebellar testing revealed prominent dysmetria of the upper and lower extremities as well as dysdiadochokinesia. His gait was ataxic and spastic. A postural and kinetic tremor of the upper extremities was present.

Longitudinal ophthalmological examination documented the presence of myopia and never revealed any sign of cataracts.

### Patient 2 (II.2)

The second patient is the proband's younger sister. She was first seen in our neurogenetics clinic at the age of 11 years. Her disease presentation was similar but much milder than what had been observed for her brother; hypotonia was first noticed at the age of 14 months. She also had motor developmental delay: she walked at 5 years of age. Her intellectual functions have not been formally assessed but at the time of her latest evaluation at the age of 21 years, she was in a regular class at school and functioning at the same level of her peers.

At the age of 21 years, her neurological examination showed diffuse mild hypotonia and hyperreflexia along with cerebellar features, i.e., dysarthria, dysmetria, gait ataxia, abnormal saccadic movements, and horizontal gaze-evoked nystagmus. The muscular strength was normal and no myopathic signs were noticed. The patient has been followed for myopia since childhood and no cataracts have ever been detected. She underwent a muscular ultrasound of the right and left quadriceps and tibial muscles that showed normal size and echogenicity.

The MRI of the brain performed at the age of 21 years revealed the presence of severe cerebellar atrophy associated with abnormally high T2 signal at the level of the dentate nuclei and posterior aspect of the pons (Fig. 1).

## Methods

Both unaffected parents and the two affected children gave informed consent, after which clinical information and blood were collected. Genomic DNA was extracted from blood using the Puregene DNA kit, following the manufacturer's protocol (Gentra System, USA). WES was performed on the unaffected father and two affected children (Fig. 2, Individuals I-1, II-1, and II-2). Standard manufacturer protocols was performed for target capture with the Agilent SureSelect All Exon 50 MB (V3) exome enrichment kit and sequencing of 100 bp paired end reads on Illumina HiSeq 2000. For each sample, single nucleotide variants (SNVs) and short insertions and deletions (indels) were called using samtools mpileup with the extended base alignment quality (BAQ) adjustment (−E), and were then quality filtered to require at least 20 % of reads supporting the variant call. Variants were annotated using both Annovar and custom scripts to identify whether they affected protein coding sequence, and whether they had previously been seen in dbSNP132, the 1000 genomes dataset (Feb2012), or in approximately 1052 WES sequenced at our center. All interesting variants were confirmed by traditional Sanger Sequencing in the four family members.

## Results

We identified two heterozygote mutations in the *SIL1* gene in both affected cases (Fig. 2). After Sanger sequencing of all family members, we establish that the first mutation c.740C T, p.A247V was transmitted by the father. The second mutation c.783G C, p. Q261H was inherited from the mother. These two mutations are not found in any public database

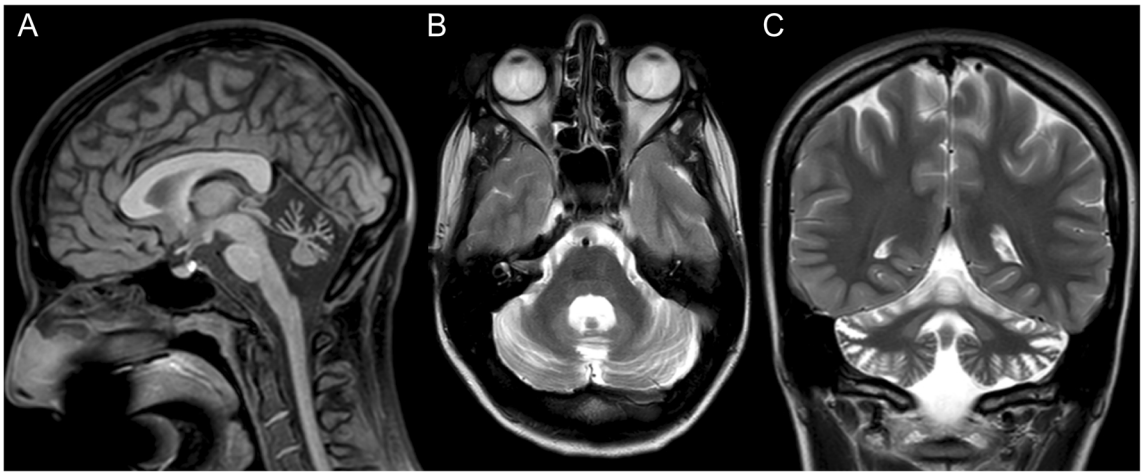



**Fig. 1** Brain MRI of Patient 2 (II.2). Sagittal T1 weighted image (**a**), axial (**b**) and coronal (**c**) T2 weighted images showing severe vermian and hemispheric cerebellar atrophy and mild T2-hyperintense signal at the level of the dentate nuclei and posterior pons

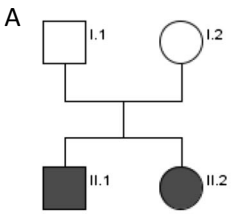
(ESV5400, dbSNP, or OMIM). Damaging score calculations in Polyphen2 predict that the two mutations are probably damaging, with the highest score of 1.00.

SIL1 is a glycoprotein in the endoplasmic reticulum (ER) [9] that interacts with ATPase domain of heat shock proteins and enhances nucleotide exchange [9]. Mutations in *SIL1* gene probably cause ER perturbation leading to protein accumulation, ER stress and finally neurodegeneration.

## Discussion

This case study offers another example of an instance where WES established the genetic cause of disease in a recessive family. Using a WES approach we identified two novel mutations in *SIL1* that cause cerebellar ataxia and atrophy [10]. While the clinical phenotype helped to orient the diagnosis, the differential diagnosis for cerebellar ataxia remained broad; hence, we opted to use a WES approach to identify the specific genetic defect leading to the disease.

The European Federation of Neurological Societies (EFNS) and European Neurological Society (ENS) guidelines to establish the genetic cause in this family with a recessive ataxia suggest a three-step approach for mutation analysis [3]. Firstly, the *FRDA* gene should be screened since it is the most common cause of autosomal recessive ataxia and can present in an atypical fashion. Secondly, in the event no *FRDA* mutations are found, a subsequent screening should be done for




C

| Individual | Clinical status | Mutation (Amino acid change) |
|---|---|---|
| I.1 | Unaffected | c.740 C>T, (p.A247V) |
| I.2 | Unaffected | c.783G>C, (p.Q261H) |
| II.1 | Affected | c.740 C>T, (p.A247V) + c.783G>C, (p.Q261H) |
| II.2 | Affected | c.740 C>T, (p.A247V) + c.783G>C, (p.Q261H) |

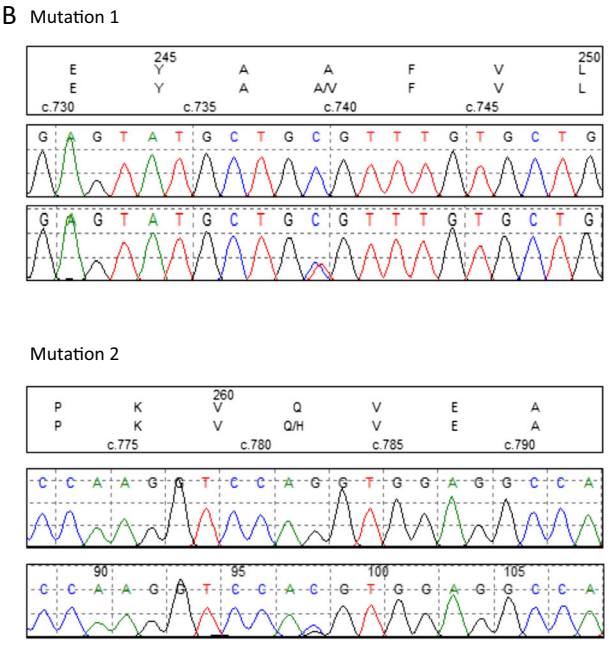



**Fig. 2** (**a**) Pedigree. (**b**) Sequences traces for each mutation found in *SIL1* gene. (**c**) Co-segregation of the mutations within the family members

*SACS*, *POLG*, and *APTX*. Finally, a muscle or skin biopsy should be obtained for biochemical enzyme deficiency analysis [3]. We found the *FRDA* gene to be normal and opted to directly perform whole exome sequencing (WES), rather than screening the small number of genes of the second step.

*SIL1* mutations cause MSS, an early-onset multisystem disorder characterized by cerebellar ataxia and atrophy in a more complex clinical picture that includes also cataracts and myopathy. Krieger et al. identified *SIL1* mutations in approximately 60 % of all patients with the full-blown phenotype of MSS—cerebellar ataxia, cataracts and myopathy—and concluded that this triad strongly suggest the presence of *SIL1* mutations [4].

Our patients do not fulfill the clinical diagnostic criteria for MSS, as myopathy and cataracts are absent in both of them. In addition, the disease onset is later and the course is milder our two patients than in the more classic form of MSS. Therefore, it was unexpected to identify *SIL1* mutations in our patients, as *SIL1* mutations were reported to be rare (2.7 %) in MSS patients that lacked one or more of the core features [4]. Interestingly, the woozy mouse model has a mutation that disrupts *SIL1* and shows a late-onset cerebellar ataxia and Purkinje cell loss, without extraneurological features [11], similar to the phenotype observed in our subjects. Therefore, we suggest that screening of *SIL1* mutations should be considered in patients with cerebellar ataxia and atrophy, even without other associated features.

Finally, this report represents another example that rare syndromes can be found in any population; *SIL1* mutations had not been previously reported in French-Canadian ataxia cases.

**Acknowledgments** We are grateful to the patients and their parents for their participation in this study. A.N. holds a Doctoral Award—Frederick Banting and Charles Best Graduate Scholarship by the Canadian Institutes of Health Research (CIHR) and a post-doctoral fellowship from the Fonds de Recherche du Québec en Santé (FRQS). R.L.P. received the Preston-Robb fellowship from the Montreal Neurological Institute and a doctoral scholarship from the Fonds de Recherche du Québec en Santé (FRQS). G.B. has received a Research Scholar Junior 1 award from the Fonds de Recherche du Québec en Santé (FRQS). G.A.R. holds a Canada Research Chair for the Genetics of Brain Diseases and is supported by the RMGA and the Canadian Institutes of Health Research. This work was selected for study by the FORGE Canada Steering Committee, consisting of K. Boycott (University of Ottawa), J. Friedman (University of British Columbia), J. Michaud (University of Montreal), F. Bernier (University of Calgary), M. Brudno (University of Toronto), B. Fernandez (Memorial University), B. Knoppers (McGill University), M. Samuels (University of Montreal), and S. Scherer (University of Toronto). We would also like to thank the McGill University and Genome Quebec Innovation Center.

**Conflict of interest** The authors declare that they have no competing interests.

## References

1. Jayadev S, Bird TD (2013) Hereditary ataxias: overview. Genet Med: official J Am College Med Genet 15(9):673–683
2. Martineau L, Noreau A, Dupre N (2014) Therapies for ataxias. Curr Treat Options Neurol 16(7):300
3. van de Warrenburg BP, van Gaalen J, Boesch S, et al. (2014) EFNS/ENS consensus on the diagnosis and management of chronic ataxias in adulthood. Eur J Neurol 21(4):552–562
4. Krieger M, Roos A, Stendel C, et al. (2013) SIL1 mutations and clinical spectrum in patients with Marinesco-Sjogren syndrome. Brain 136(Pt 12):3634–3644
5. Horvers M, Anttonen AK, Lehesjoki AE, et al. (2013) Marinesco-Sjogren syndrome due to SIL1 mutations with a comment on the clinical phenotype. Eur J Paediatr Neurol 17(2):199–203
6. Lagier-Tourenne C, Tranebaerg L, Chaigne D, et al. (2003) Homozygosity mapping of Marinesco-Sjogren syndrome to 5q31. Eur J Hum Genet 11(10):770–778
7. Anttonen AK, Mahjneh I, Hamalainen RH, et al. (2005) The gene disrupted in Marinesco-Sjogren syndrome encodes SIL1, an HSPA5 cochaperone. Nat Genet 37(12):1309–1311
8. Anheim M, Fleury M, Monga B, et al. (2010) Epidemiological, clinical, paraclinical and molecular study of a cohort of 102 patients affected with autosomal recessive progressive cerebellar ataxia from Alsace, Eastern France: implications for clinical management. Neurogenetics 11(1):1–12
9. Senderek J, Krieger M, Stendel C, et al. (2005) Mutations in SIL1 cause Marinesco-Sjogren syndrome, a cerebellar ataxia with cataract and myopathy. Nat Genet 37(12):1312–1314
10. Biesecker LG, Green RC (2014) Diagnostic clinical genome and exome sequencing. N Engl J Med 370(25):2418–2425
11. Zhao L, Longo-Guess C, Harris BS, Lee JW, Ackerman SL (2005) Protein accumulation and neurodegeneration in the woozy mutant mouse is caused by disruption of SIL1, a cochaperone of BiP. Nat Genet 37(9):974–979