**Supplement**

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**e-Methods.** **Genetic analyses.**

Whole genome sequencing (WGS) was conducted at Genome Quebec (Montreal, Canada) on samples from 125 Canadian patients who were diagnosed with ALS at Sunnybrook Health Sciences Centre (Toronto, Canada). In brief, WGS was performed using the NovaSeq 6000 (Illumina) PE150, with a minimum average sequencing depth of 30; 1 ug DNA was used for PCR-free library preparation using the NxSeq® AmpFREE Low DNA Library Kit (Lucigen). The raw fastq data were aligned to UCSC hg19 genome reference to generate SAM and BAM files, using the BWA (<http://bio-bwa.sourceforge.net>) and SAMTOOL (<http://samtools.sourceforge.net>). The GATK pipeline (<https://gatk.broadinstitute.org/hc/en-us>) was used to call variants.

Whole exome sequencing (WES) was conducted on samples from 162 French-Canadian ALS patients from the Montreal Neurological Institute (Montreal, Canada). HiSeq2000 and HiSeq2500 sequencing was performed with average coverage of 145.11X. Libraries were generating using Agilent SureSelect Human all Exon v4 kits. Raw fastq were aligned to the GRCh37 reference genome using bwa mem. GATK version 3.7 was used to call variants and joint genotype the cohort. Filters of GQ > 30 and DP > 30 were applied to sample genotypes. Variants were annotated for functional outcomes and population frequency using ANNOVAR.1

We also analyzed *GBA* variants in 4,366 ALS patients and 1,832 age- and sex-matched controls from the Project MinE WGS dataset.2,3

**e-Results. Patients.**

**Case 1**

A 52-year-old French-Canadian male with GD1 presented with a 1-year history of falls and muscle cramps in his neck, back, and limbs, as well as a 6-month history of involuntary flickering of limb muscles. He denied any bulbar symptoms, significant neck or back pain, numbness, tingling, bowel or bladder difficulties. He did not report symptoms of pseudobulbar affect. There was no history suggestive of REM-behaviour sleep disorder. This occurred on a background of known GD1 diagnosed upon genetic testing at age 2 (prompted by diagnosis of the same condition in his older sister), which revealed compound heterozygous mutations (N370S/W378G) in the *GBA* gene. His GD-related features included splenomegaly, thrombocytopenia, fatigue, and recurrent upper respiratory infections. His past medical history was otherwise significant only for hypertension (aortic root dilatation excluded). He had previously participated in a clinical trial with taliglucerase, an enzyme replacement therapy (ERT) and was later maintained on the ERT velaglucerase alfa, as well as candesartan and vitamin D supplementation.

There was a strong family history of neurodegenerative disorders, but no known ALS (**Figure e-1**). His mother had late-onset dementia diagnosed as Alzheimer’s disease. His father had a diagnosis of late-onset PD, and a first maternal cousin once removed had PD with severe mobility issues and died at age 60. Possible PD was also reported in his paternal grandmother. His paternal aunt had been reported to have DLB prior to death in her 90s. Frontotemporal dementia was reported in his maternal uncle, and late-onset dementia was reported in one maternal aunt (possible AD) and one maternal uncle. Of his four siblings, one sister had GD1, two brothers were carriers, and one brother was a non-carrier. None of his siblings had any known neurological complaints. He had two children without symptoms.

Clinical examination showed mild cognitive deficits; Montreal Cognitive Assessment (MoCA) score was 25/30 with points lost on delayed recall and language domains. Cranial examination was normal except for moderate facial masking. He had diffuse wasting of limb muscles, particularly of the right first dorsal interosseous (FDI). Fasciculations were evident in the thoracic paraspinals and limbs diffusely. No tongue or facial fasciculations were noted. There was a fine action tremor of the hands, worse on the left. Tone was normal in all four extremities. Manual muscle testing showed severe weakness on foot dorsiflexion (grade 0/5 on the right, grade 1/5 on the left), and great toe extension (grade 0 bilaterally). Reflexes were absent in the upper extremities, 3+ at the knees, and 1+ at the ankles bilaterally. Plantar response was extensor on the left and flexor on the right. Sensation to pain, temperature, and vibration was intact in the upper extremities but reduced in the feet. Proprioception was intact distally in all four extremities. There was no appendicular or gait ataxia. He had a high-steppage gait and was unable to walk on heels but was able to walk on toes. Postural stability was intact. He had minimal signs of parkinsonism (e.g., masked facies) but no rigidity, bradykinesia, rest tremor, or postural instability to support a diagnosis of PD or an atypical parkinsonian disorder. Electromyography (EMG) and nerve conduction studies (NCS) showed evidence of acute and chronic neurogenic changes in proximal and distal muscles of three limbs as well as in thoracic paraspinal muscles, in keeping with a motor neuronopathy. No EMG abnormalities were evident in the cranial segment. There was also evidence of a mild length-dependent sensory polyneuropathy. Abnormalities on sensory nerve studies were isolated to a mild decrease in amplitude of the right sural sensory response, in contrast to the diffuse and more marked abnormalities seen on motor NCS and EMG.

MRI of the brain was normal. Total spine MRI showed mild degenerative changes of the vertebral column without any evidence of compression of nerve roots or spinal cord. Mild diffuse atrophy of the thoracic spinal cord and conus were noted, in the absence of any signal abnormalities.

Serologies were negative for West Nile Virus (WNV), Lyme disease, human immunodeficiency virus (HIV), and human T-lymphotropic virus (HTLV). Syphilis testing was negative. Thyroid-stimulating hormone (TSH) levels were normal. Testing for rheumatoid factor, anti-nuclear antibodies, and anti-DNA antibodies was negative. Beta- hexosaminidase assay testing was normal, excluding Tay-Sachs disease. Creatine kinase was minimally elevated at 256 U/L. He had an IgG-lambda paraprotein at a concentration of 8.2 g/L, with a normal kappa/lambda ratio.

Thrombocyte count was reduced at 87 x 109/L, stable from prior testing. Total bilirubin, hemoglobin and white blood cell count were normal. MRI of the abdomen and pelvis showed splenomegaly, with multiple T2 hyperintense splenic nodules, stable from previous imaging.

The patient was diagnosed with clinically probable laboratory-supported ALS, based on the revised El Escorial criteria. He was treated with riluzole. Genetic testing with panel for genes associated with ALS/frontotemporal dementia (FTD) revealed a heterozygous sequestosome 1 (*SQSTM1*) variant c.1129C>G, which is predicted to result in the amino acid substitution L377V. Parental testing showed the *SQSTM1* variant did not segregate with disease. He had a normal number of hexanucleotide *C9orf72* repeats.

**Case 2**

An Israeli Ashkenazi Jewish female patient with a long history of severe GD1 reported onset of dysarthria at age 54. She had been diagnosed with GD1 at the age of 4 years after presenting with massive hepatosplenomegaly, thrombocytopenia with bleeding tendency, and anemia. She underwent splenectomy at the same age with normalization of the cytopenias, but developed late bone complications including repeated episodes of bone crisis, pathological fractures, and bilateral osteonecrosis. She underwent hysterectomy after life-threatening postpartum bleeding following the birth of her second daughter. She was first evaluated at the Gaucher Unit at Shaare Zedek Medical Center (Jerusalem, Israel) at the age of 35. She was cachectic, dyspneic, and presented with massive hepatomegaly, peripheral cyanosis, and clubbing due to hepatopulmonary syndrome. Her GD1 diagnosis was confirmed by low enzymatic activity of the β-glucocerebrosidase, and genetic testing revealed compound heterozygous N370S/c.84dupG mutations. Low-dose ERT with imiglucerase was initiated and led to dramatic improvement of dyspnea, reduction of hepatomegaly, gradual disappearance of acrocynosis, and later improvement of clubbing. She underwent left total hip replacement at age 37. Approximately 5 years after starting ERT, she gradually developed primary-like pulmonary hypertension, treated with anticoagulation and ileomedin inhalation. By age 41, she was aphonic and was diagnosed with secondary Ortner syndrome that remained stable since then.

Neurological exam at age 55 showed hoarseness, dysarthria, and slowed speech. She had weak facial and tongue muscles, questionable tongue fasciculation, and a positive jaw jerk reflex. Ocular movements were normal. There wase no limb atrophy. Muscle tone was normal. Muscle power was full in the 4 limbs, except for 4/5 in the deltoids. There was limb hyperreflexia and bilateral extensor plantar responses. There were no sensory deficits. EMG revealed few distal fibrillations. Brain MRI and cervical CT scan were normal.

She was diagnosed with probable ALS based on El-Escorial criteria. She ultimately developed signs of clinically definite ALS, her condition rapidly deteriorated and she died at age 56.

**Case 3**

A 70-year-old female with a 1-year history of body aches and exhaustion presented with recent onset of respiratory symptoms. These were followed by proximal leg weakness with difficulties going up the stairs. Over the following 6 months, her walking difficulties progressed, and she required the use of a walker. She also reported weakness of the upper limbs, twitches in the right leg and abdominal area, and occasional abdominal spams. At age 71, wasting of the posterior paraspinal muscles was evident on lumbar MRI.

Her past medical history included bipolar disorder treated with lithium and probable fibromyalgia treated with duloxetine, acetaminophen, and nonsteroidal anti-inflammatory drugs. She also had multilevel degenerative disc disease and prior motor vehicle accidents, falls related to skiing, and vertebral fractures.

Neurological exam 19 months after onset of respiratory symptoms showed mixed upper motor neuron (UMN) and lower motor neuron (LMN) findings. There was no obvious parkinsonism or other movement disorder findings.

Her electrophysiological findings were reported to be consistent with typical ALS, without any unusual features. Lumbar MRI showed evident wasting of the posterior paraspinal muscles as well as multilevel disc degeneration, old T12 and L1 fractures, mild L3-L4 spinal stenosis, bilateral L5-S1 foraminal narrowing by disc/osteophyte complex, without evidence of compression of the conus medullaris or cauda equina.

Her laboratory tests showed elevated creatine kinase (805 U/L, reference range 38-234 U/L), normal red and total white blood cells, normal platelets, vitamin B12 levels, hemoglobin A1c, and TSH levels.

She died after BiPAP withdrawal at age 71, twenty-two months after onset of respiratory symptoms.

She had not undergone any genetic testing but she was a presumed obligate carrier of a *GBA* mutation (N370S or P236T) because her daughter had GD1 due to compound heterozygous N370S/P236T mutations in the *GBA* gene. Neither she nor her daughter’s father was of Ashkenazi Jewish descent. Her daughter was diagnosed with GD1 at age 50 based on a positive bone marrow biopsy and longstanding history of malaise, enlarged spleen, reduced platelets, and elevated ferritin levels. Her daughter was diagnosed with PD at age 51.

**![Diagram, schematic

Description automatically generated]()**

**Figure e-1. Pedigree of case 1.**

Proband is designated by the arrow. AD, Alzheimer disease; ALS, amyotrophic lateral sclerosis; DLB, dementia with Lewy bodies; FTD, frontotemporal dementia; GD1, Gaucher disease type 1; PD, Parkinson disease; SQSTM1,sequestosome 1 gene; VUS, variant of uncertain significance; y.o., years-old.

**References**

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