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IDENTICAL TWINS WITH THE *C9ORF72* REPEAT EXPANSION ARE DISCORDANT FOR ALS

Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease that could co-occur with frontotemporal dementia (FTD), characterized by early behavioral or language changes. Mutations causing ALS and FTD have been found in several often overlapping genes.¹ The most common mutation for both syndromes is a noncoding G₄C₂ expansion in *C9orf72*,^{2,3} usually ranging from hundreds to thousands of repeats.⁴ Currently it is unclear whether expansion alleles with different sizes have the same pathologic consequence, and the lower limit for pathologic repeat number has not been determined.^{4,5}

Haploinsufficiency is proposed to be one of the disease mechanisms, since *C9orf72* transcription is reduced by ~50% in expansion carriers,² possibly as a result of epigenetic modifications. Indeed, the expansion was associated with DNA hypermethylation of the CpG island 5' of the G₄C₂ repeat (in blood, brain, spinal cord), and this association was strongest in patients with familial ALS or short disease duration.⁵ Intriguingly, half of the carriers (mostly sporadic) revealed low or no methylation.

There is wide diversity of *C9orf72* phenotypes (ALS, ALS/FTD, FTD), presenting with highly variable ages at onset (20–80 years) and disease duration (a few months to decades),⁶ implying the existence of modifying factors, which could be best investigated in monozygotic (MZ) twins. We present clinical, genetic, and epigenetic findings in a pair of ALS-discordant MZ twins.

Methods. *Standard protocol approvals and patient consents.* Informed consent was obtained from all participants and the study was approved by the ethics review board of the Sunnybrook Health Sciences Centre (Toronto, Canada).

Detailed methods are available in appendix e-1 on the *Neurology*[®] Web site at Neurology.org. Briefly, PED18 family members (n = 5) were recruited from the ALS Clinic at Sunnybrook. DNA was isolated from blood and genotyped for 8 microsatellites. Southern blotting,⁴ methylation analyses,^{5,7} and *C9orf72* genotyping^{5,7} were described previously.

Mutations in *SOD1*, *FUS*, *TARDBP*, and *GRN* were excluded by Sanger sequencing in the twin with ALS. Both twins were also genotyped on the NeuroX array (Illumina, San Diego, CA) designed to study neurologic diseases.

Results. PED18 is a Caucasian Semitic family with female MZ twins (figure, A and appendix e-1 for clinical details). Twin 9104 developed symptoms of bulbar onset ALS at age 57 years. To date, her cognitive testing is normal and there is no indication of FTD symptoms. Notably, she had head trauma without losing consciousness 1 year before onset and smoked half a pack of cigarettes a day for 10 years, quitting in her late 20s. In contrast, twin 9105 remains asymptomatic at age 62 and has no history of smoking or head trauma. Her cognitive testing revealed mild abnormalities and she scored 23/30 on the Montreal Cognitive Assessment, displaying deficits in phonemic verbal fluency, abstraction, and executive function.

Monozygosity of the twins was confirmed by identical microsatellite genotypes (table e-1) and 267,607 variations on the NeuroX array, which did not reveal any mutations implicated in neurodegenerative disorders. Genotyping of *C9orf72* identified 3 heterozygous carriers of the G₄C₂ expansion (including the twins), likely inherited from the father, who developed progressive dysarthria and had a sibling with ALS. Southern blotting revealed the same 2-peak pattern of expansion species in each carrier, with the most abundant repeat sizes of ~800 and ~1,350 repeats likely due to somatic instability of the expansion (figure, B). Two methylation assays did not detect increased methylation at the 5' CpG island for any family member (figure, A, C, and D).

Discussion. Despite the identical genetic background, the twins have been ALS discordant for 5 years, which is not explained by variability in expansion size or epigenetic differences (both twins have a similar range of repeat size and no methylation at the CpG island 5' of the G₄C₂ repeat). Notably, mild deficits in verbal fluency and executive function were only observed in the twin unaffected by ALS, which could be the first signs of FTD. The driving force behind the phenotypic split (ALS vs FTD) in

Supplemental data
at Neurology.org

Figure Results of *C9orf72* genotyping, Southern blotting, and methylation analyses for PED18

(A) The PED18 pedigree with *C9orf72* genotype beneath the individual ID (exp = expansion). Age at time of examination is shown in the upper right corner. The number of methylated CpGs is shown to the right of the gel image beneath bisulfite PCR (BSP). Affected individuals are shown as filled symbols. A slash indicates deceased persons. Sex is masked to protect family confidentiality. ALS = amyotrophic lateral sclerosis. (B) Southern blot analysis using genomic DNA from blood confirmed the presence of expansions in 9104, 9105, and 9106, and its absence in 9820 and 9273. The expansion size is similar in the 3 carriers. The molecular weight marker is shown on the left side of the blot (POS CON = positive control for the expansion). (C) Representative chromatograms of the bisulfite sequencing results for the CpG island 5' of the G_4C_2 repeat are shown, containing the first 3 out of 26 studied CpG sites (black squares at the top of the sequence diagram), while all non-CpG C (blue squares) were successfully converted to T. (D) Schematic drawing of the 26 studied CpG sites. The monozygotic twins (9104 and 9105) carrying the expansion and noncarriers (9820 and 9273) showed no methylation at the investigated CpG island. Individual 9106 (expansion carrier) showed a low methylation level with 3 methylated CpG sites.

expansion carriers is unknown, and if the second twin does develop FTD, it could argue in favor of environmental vs genetic modifiers. Of note, both twins have had similar environmental exposures; however, the twin with ALS was a smoker and had a head trauma.

The identical genetic background of the MZ twins, and similarities in both methylation level and repeat size, might argue against a genetic modifier. However, our study was limited to the investigation of a single family and larger twin studies could

help further understand the clinical diversity of *C9orf72*-related disease.

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Author contributions: Dr. Xi was responsible for the genetic and methylation studies of *C9orf72* including data analyses. Dr. Yunusova was involved in sample collection and patient characterization. Dr. Van Blitterswijk employed Southern blotting techniques to determine repeat sizes, which included data analysis,

interpretation of results, and revision of the manuscript. Dr. Ghani was responsible for analysis of NeuroX array. Drs. Liang, Moreno, and Sato were involved in sample preparation and sequencing of ALS genes. Dr. Dib assisted in experimental design and data interpretation. Dr. Singleton was responsible for analysis of NeuroX array. Dr. Robertson assisted in experimental design and data interpretation. Dr. Rademakers supervised experiments and obtained funding. Dr. Zinman was responsible for obtaining clinical findings. Dr. Rogaeva conceived and supervised the project and drafted the manuscript with Dr. Xi and Dr. Zinman. All authors critically reviewed and approved the final manuscript.

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patent on methods to screen for the hexanucleotide repeat expansion in the C9orf72 gene. L. Zinman reports no disclosures relevant to the manuscript. E. Rogaeva receives research support from the W. Garfield Weston Foundation and Ontario Research Fund. Go to Neurology.org for full disclosures.

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DRAMATIC RESPONSE OF A BRAF V600E-MUTATED PRIMARY CNS HISTIOCYTIC SARCOMA TO VEMURAFENIB

Histiocytic sarcoma (HS) is classified within the malignant histiocytic disorders.¹ Involvement of isolated CNS in HS, termed primary CNS-HS (PCNS-HS), is characterized by a proliferation of cells in the monocyte/macrophage lineage within the CNS.^{2–6} This case report documents the clinical, biological, pathologic, and radiologic spectrums of PCNS-HS.

This single observational study without controls provides Class IV evidence.

Case report. A 40-year-old man received consultation for memory impairment, paroxysmal right-sided hemibody paresthesia, and neck pain. A left temporal mass was detected by brain MRI (figure, Aa–c); therefore, a stereotactic biopsy of the lesion was performed. The pathologic examination (K.M., J.-F.E., and Dr. Frederic Charlotte) indicated HS according to the WHO classification (figure, Ba–f).

Diagnoses of glioma, germinoma, lymphoma, and metastatic tumors (melanoma and carcinoma) were excluded. The tumor cells exhibited the following attributes: (1) diffusely positive for CD68 and CD4; (2) focally positive for CD163 and S100; and (3) negative for glial fibrillary acid protein, OLIG2, PLAP, CD30, CD45, CD20, CD79a, CD3, HMB45, MelanA, and cytokeratins.

A comprehensive workup (CT scan, ¹⁸FDG PET scan, and spinal cord MRI) had normal results, and the patient was doing well with analgesic drugs. Two weeks later, he experienced impaired consciousness, and MRI revealed acute, nonobstructive hydrocephalus (figure, Ad–f). Steroid therapy was begun, and a lumbar puncture was performed. CSF analysis found increased protein (1.76 g/L), decreased glucose (0.7 mM), 31 leukocytes/mL, 5 erythrocytes/mL, and no organisms. A ventriculoperitoneal CSF shunt was inserted, leading to clinical improvement. Pathologic review of the CSF revealed tumor cells (figure, Bg). Sequencing of tumor DNA, extracted from a tumor sample obtained by stereotactic biopsy, revealed a heterozygous *BRAF* V600E