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CORRESPONDENCE

C9orf72-specific phenomena associated with frontotemporal dementia and gastrointestinal symptoms in the absence of TDP-43 aggregation

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The most common genetic cause of frontotemporal dementia is the expanded hexanucleotide (GGGGCC) repeat insertion in a non-coding promoter region of the chromosome 9 open reading frame 72 *(C9orf72)* gene [4, 13]. Nearly all patients who carry the *C9orf72* expansion show well-developed TAR DNA-binding protein 43 (TDP-43) inclusion pathology at autopsy, and TDP-43 has been considered a key driver of neurodegeneration based on human clinicopathological correlation approaches [7]. Scattered case reports describing pre-symptomatic *C9orf72* expansion carriers suggest, however, that *C9orf72*-specific phenomena such as dipeptide repeat (DPR) proteins and RNA foci can be observed in the absence of or even preceding TDP-43 inclusions [11, 15]. One previous report of a patient with behavioral variant FTD (bvFTD) suggested that focal degeneration could occur in brain regions lacking TDP-43 aggregation, but to date no patient with symptomatic C9orf72-associated "probable" FTD has lacked TDP-43 inclusions altogether [11]. Likewise, although DPRs have been identified in the testis, there has been no evidence of symptomatic DPR aggregation outside the central nervous system (CNS) [2].

Here, we report a 65-year-old woman who developed slowly progressive behavioral changes over at least 1 year. She withdrew from friends, became emotionally labile, and grew disinterested in her hobbies. At times, she struggled to cook for herself. In parallel, she developed hyposmia, compulsive binge-eating, nausea, vomiting, loose stools, bowel incontinence, and a 20-pound weight gain. Classical disinhibition and compulsive behaviors were lacking. She had no known family history of neurodegenerative disorders. Her mother was alive and cognitively normal; but her father, who suffered from a stroke, was described as having a mental illness at the time of his death at 71 years of age. Her neurologic exam demonstrated mildly increased tone in her arms, rare myoclonic jerks, and a slightly reduced stride length.

Her medications (see Supplemental Table 1) were without recent changes. Extensive laboratory testing (see Supplemental Table 2) failed to reveal a cause of her gastrointestinal or behavioral symptoms. A brain magnetic resonance image (MRI) without contrast (Fig. 1a, Supplemental Fig. 1) showed moderate, distributed atrophy found most prominent in the anterior insula, medial frontal, anterior temporal, and parietal lobes. A routine electroencephalogram showed mild slowing of the posterior dominant rhythm. Esophagogastroduodenoscopy demonstrated moderate esophagitis, a non-obstructive Shatzki ring, and mild non-erosive gastritis, consistent with gastroesophageal reflux disease, but considered insufficient to produce her symptoms. Gastric biopsies were negative for *H. pylori*. Duodenal biopsies were negative for histologic or immunophenotypic evidence of Celiac or Whipple's disease. A colonoscopy with terminal ileum biopsies was likewise unremarkable.

Over the next 2 years, the patient developed worsening parkinsonism, dysphagia, and intractable vomiting. She could manage only basic household tasks, requiring assistance with driving, shopping, and managing her finances.

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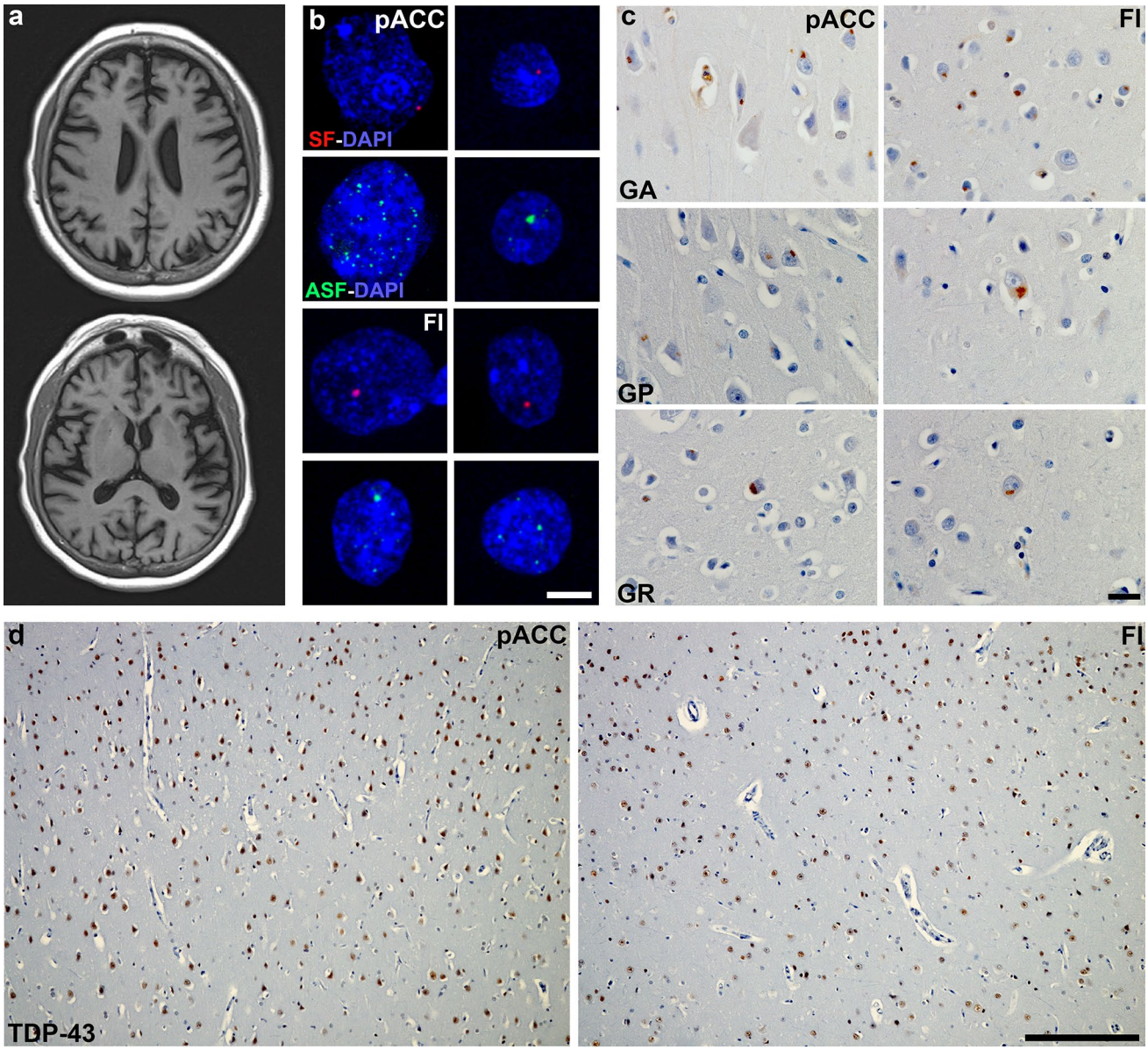

Fig. 1 A *C9orf72* expansion carrier without TDP-43 inclusion pathology. **a** Axial slices from the patient's T1-weighted MRI scan show moderate to severe atrophy, most prominent in the insular, frontal, and parietal brain regions. **b–d** Representative images showing the presence of *C9orf72*-specific phenomena such as RNA foci and DPR inclusions in affected brain regions, including the pACC and FI. TDP-43 immuno staining showed normal nuclear TDP-43 staining and an absence of TDP-43 inclusions. Scale bars represent 5 µm (**b**), 25 µm (**c**), and 250 µm (**d**). *pACC* pregenual anterior cingulate cortex, *FI* frontal insula, *SF* sense RNA foci, *ASF* antisense RNA foci

Neuropsychological testing demonstrated moderate difficulties in executive functioning and processing speed, with relatively preserved visuospatial skills and episodic memory. Because of her progressive behavioral decline, early loss of interest in friends and hobbies (i.e. apathy), changes in eating behavior, executive-predominant neuropsychological profile, and frontal-predominant atrophy on MRI, she met criteria for probable bvFTD [12]. Genetic testing later revealed a large GGGGCC expansion (> 1000 repeats) in *C9orf72*, leading to a diagnosis of definite bvFTD by international consensus criteria [12]. In her final weeks, she could not eat without vomiting. Three years after her first symptoms, she woke up one morning, made oatmeal, and went to the bathroom nauseated. After several rounds of emesis, she collapsed and died, apparently due to aspiration.

A general autopsy was performed. Gross examination of the gastrointestinal (GI) tract was unremarkable. The fresh brain weighed 1190 grams. Gross neuropathological

assessment revealed mild to moderate volume loss of the frontal and temporal lobes bilaterally. Sections stained for hematoxylin and eosin were rated for microvacuolation and astrogliosis (Supplemental Table 3); overall, these changes were absent or mild, with only selected bvFTD-susceptible regions showing evidence of mild neurodegeneration. There were scattered microinfarcts and siderocalcinosis in the globus pallidus, but the substantia nigra showed normal findings. Immunohistochemical studies revealed sparse to frequent diffused amyloid-beta plaques in the neocortex (Thal Phase 1), tau neurofibrillary pathology involving the entorhinal region, hippocampus, and amygdala (Braak neurofibrillary tangle stage 3), and no Lewy body disease on alpha-synuclein immunostaining. Immunostaining for TATA-binding protein-associated factor 15 (TAF15), one of the most sensitive antibodies for detecting FTLD-FET

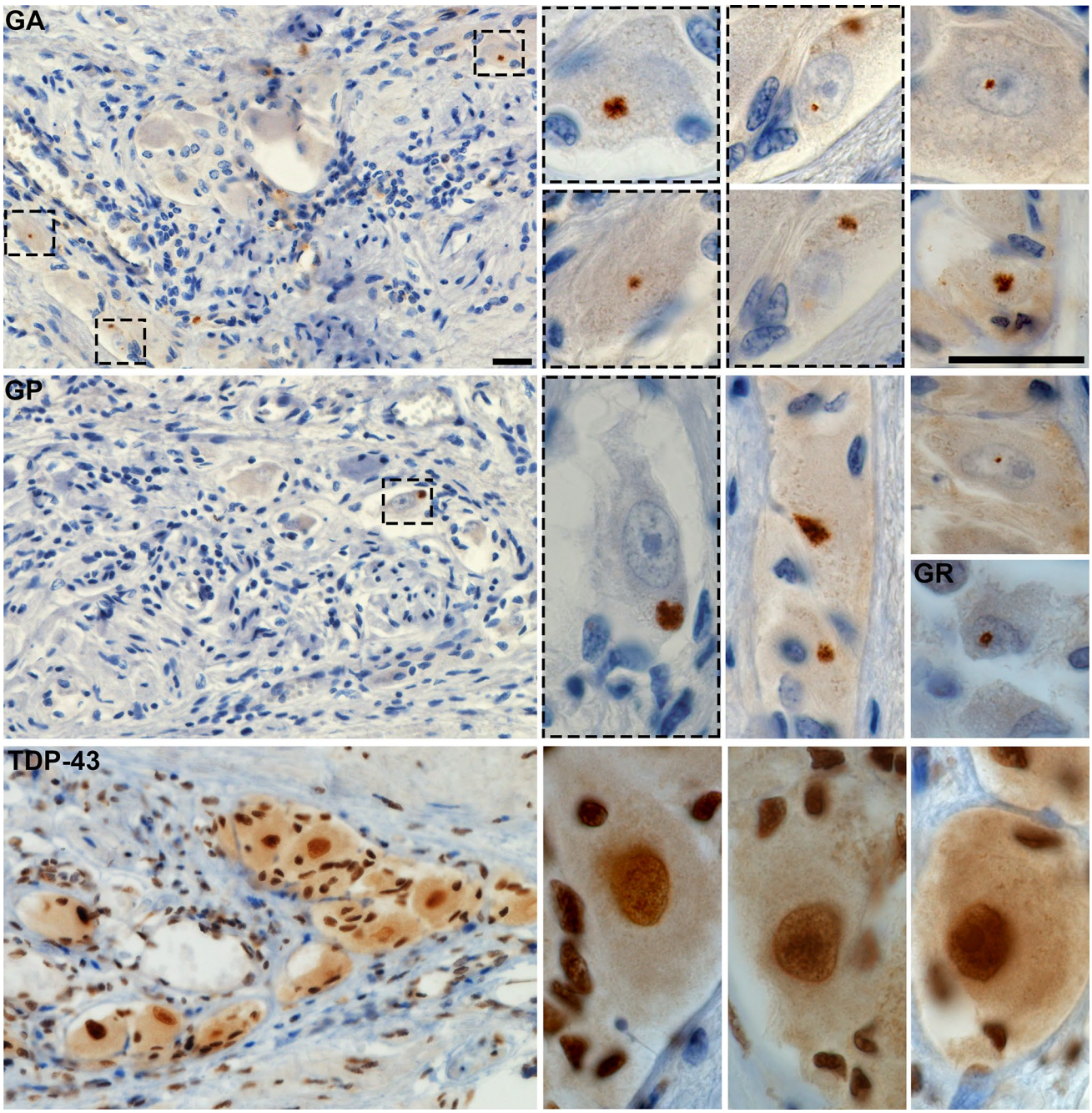

Fig. 2 DPR inclusions are present in the enteric nervous system. Poly-GA, -GP, and -GR DPR inclusions are shown in the enteric neurons of the myenteric (Auerbach's) plexus of the gastroesophageal junction or sigmoid colon. In these regions, TDP-43 immunoreactivity shows normal nuclear staining of the enteric neurons. Scale bars represent 25 µm

inclusions, showed no inclusion pathology with normal nuclear TAF15 staining [10]. Given the known *C9orf72* expansion, we performed fluorescence in situ hybridization (FISH) for sense and antisense repeat RNA foci, which showed foci of both types in selected areas (Fig. 1b). Immunohistochemistry for each of the five DPRs revealed widespread DPR inclusions throughout the brain (Fig. 1c, Supplemental Table 3). Poly-GA was the most abundant DPR, while poly-PA and poly-PR were the least abundant. The distribution of DPRs was consistent with previous studies [3, 14]. Immunostaining for p62 showed that most neuronal cytoplasmic inclusions were perinuclear, with a stellate morphology resembling DPR inclusions (Supplemental Fig. 2). Immunostaining for full-length TDP-43 showed normal nuclear staining, without inclusions, in all 45 CNS regions evaluated (see Fig. 1d, Supplemental Table 3, Supplemental Fig. 3), except for 3–4 scattered neuronal cytoplasmic inclusions in the amygdala, a finding observed in 30–50% of cognitively normal persons of the patient's age [16].

To determine whether the patient's GI symptoms might relate to *C9orf72*-associated pathological features, we performed immunohistochemistry for the five poly-dipeptides on tissue sections from several segments of the GI tract (Fig. 2, Supplemental Table 3). Neuronal cytoplasmic inclusions chiefly composed of poly-GA and –GP were observed in neurons occupying the myenteric (Auerbach's) plexus of the gastroesophageal junction and sigmoid colon (Fig. 2). Meissner's plexus was free of inclusions. No neurons showed TDP-43 aggregation or depletion. Numerous attempts to perform FISH for repeat RNA foci on enteric samples were unsuccessful due to tissue non-adherence. Hematoxylin and eosin-stained sections throughout the GI tract showed no clear evidence of vacuolation or neuronal loss.

The present case demonstrates that the *C9orf72* expansion can be associated with: (1) probable bvFTD in the absence of FTLD-TDP and (2) severe GI symptoms accompanied by *C9orf72*-specific phenomena within the enteric nervous system.

Patients with *C9orf72*-FTD showing limited TDP-43 inclusion pathology have been reported, but to our knowledge, the present case is the first to show significant brain atrophy in the near complete absence of TDP-43 inclusions [15]. Prevailing FTLD nomenclature fails to accommodate the patient's findings, because she does not have FTLD-TDP, and FTLD with ubiquitin proteosomal system inclusions (FTLD-UPS) is intended for use, when the protein found within the ubiquitinated inclusions is not known [8]. We therefore offer the following terminology as a temporary way to describe the present case and others like her: *C9orf72*-associated FTLD without TDP-43 aggregation.

Although some studies have widely surveyed non-CNS tissues for DPR inclusions, these studies have not included the GI tract or patients with prominent GI symptomatology [2, 9]. Patients with bvFTD often have profound alterations in eating behavior, but we are not aware of prior cases in which GI dysregulation was the most disabling feature [1]. Interestingly, although our patient showed no apparent TDP-43 pathological changes, GI complications have been observed in transgenic mice with human mutant (A315T) TDP-43 [5]. The mice exhibited dilation of the cecum and terminal ileum, and histopathological analysis revealed vacuolated myenteric plexi with TDP-43 aggregates in associated ganglion cells [5, 6]. Murine models of *C9orf72* disease have not, to our knowledge, shown such findings, but the present case adds enteric neurons to the list of nervous tissues vulnerable to *C9orf72*-specific phenomena.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest relevant to this article to disclose.

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