INTRODUCTION

Dravet syndrome (DS) is a developmental and epileptic encephalopathy mainly caused by a loss-of-function mutation in one allele of *SCN1A*, which encodes the voltage-gated sodium channel $Na_v1.1$., widely expressed in fast-spiking inhibitory GABAergic interneurons, leading to brain hyperexcitability. Disease onset occurs in the first year of life, followed by progressive cognitive dysfunction. Neuropathological studies of patients with DS are scarce, and their findings are inconsistent (1, 2, 3, 4). Most of these studies focus on pediatric ages and often do not have a genetic confirmation. Our work describes the clinical findings of a family presenting four members (three sisters and the father) harbouring a missense SCN1A pathogenic variant in exon 20. The variant had been previously described, but in sporadic cases and with *de novo* occurrence (5). The present study also shows neuropathological findings of the father, with a concomitant diagnosis of Alzheimer's disease (AD).

MATERIALS & METHODS

Clinical characterisation of the family, genetic testing results and neuropathological study are presented. The patients' old medical records, family trees, thorough anamnesis and neurological examinations were analysed. Genetic testing was performed in 2012 with Sanger sequencing of the SCN1A gene in the three sisters (since DS was considered the most likely diagnosis, and Sanger sequencing was the most cost-effective tool at that time), and in postmortem brain tissue in the father in 2022. The neuropathologic examination was performed according to standardized protocols at the Neurological Tissue Bank (NTB) of Hospital Clínic-IDIBAPS in Barcelona, Spain (6) and as internationally recommended (7). In addition to regular assessments, 3 researchers independently screened for findings related to epilepsy (such as hippocampal sclerosis, cortical architectural alterations, or neuronal heterotopias) in the HE staining, and the results were discussed jointly.

RESULTS

Clinical cases

Three sisters were referred to the Epilepsy Unit of Hospital Santa Creu i Sant Pau (Barcelona) in 1,999, at the age of 25, 31 and 34 respectively, with the previous diagnosis of Lennox-Gastaut syndrome (LGS), based on the presence of epilepsy and intellectual

disability (ID). Birth was normal, but they started having prolonged febrile seizures between 6 and 12 months of age, approximately monthly. Subsequently, a psychomotor regression was observed, and multiple seizures per day appeared (bilateral tonic-clonic, tonic, absences, motor focal and myoclonic seizures), febrile or not. The oldest sister had the most severe disease, suffered from seizures throughout all her lifespan, severe ID and finally wheel chair dependency. She died at 52 because of hypercapnic respiratory insufficiency due to repeated respiratory infections. The middle sister had a moderate disease, with persistent seizures although treatment with phenobarbital -PB- and valproic acid -VPA-. She underwent callosotomy at 18 years old, with a substantial change in tonic seizures frequency. In adulthood, the addition of topiramate (TPM) to VPA and clobazam -CLB- improved significantly seizure control, and she is seizure free since 2016. Otherwise, she has moderate ID and behavioural problems, treated with antipsychotics and antidepressants. The youngest sister has the mildest disease, with a good response to callosotomy at 12 years old, remaining with monthly catamenial bilateral tonic-clonic seizures until 2018, when treatment with TPM was added to VPA and CLB, being seizure free since then. She has mild to moderate ID, without significant behavioural problems. Noteworthy, they had ataxic gait during childhood, whereas in adulthood both surviving sisters started with parkinsonian symptoms, including slowness of movements and crouched gait [Video].

Regarding the current diagnostic tests, old EEGs in both sisters showed generalized epileptiform activity (EA), whereas recent EEGs showed intermittent diffuse slowing. MRI presented mild global atrophy in both cases, apart from callosotomy-associated changes.

In 2012, genetic testing was conducted, and all three sisters showed a missense pathogenic variant in *SCN1A* p.Gly1332Glu (c.3995G>A) in exon 20.

Regarding family history, the paternal path showed several cases of epilepsy (with different degrees of severity) and psychiatric problems, but without ID [Fig 1]. We were not able to obtain more information from the paternal path, since they were living abroad, without contact with the maternal path.

The father was diagnosed with apparent generalized epilepsy in childhood, but he was seizure-free without antiseizure medication until the age of 50 years, when bilateral tonic-clonic seizures returned and treatment with VPA was started, with good response. Although significant behavioural problems all his lifespan, his intellect was intact, and he could finish two degrees. When he was 60 years old, he started experiencing memory

complaints, and was diagnosed with AD. He passed away ten years later and donated his brain to the Clínic-IDIBAPS NTB. The neuropathological study showed extensive neurofibrillary and amyloid pathology (Braak and AD Braak stage VI and Thal phase 5) and frequent neuritic plaques in the cortex (CERAD C), confirming the diagnosis of AD (High AD neuropathological change A3B3C3) (7) [Fig. 2]. Additional morphological changes that could be related to epileptic pathology were not detected.
In 2022, DNA was obtained from the cerebral tissue of the father, and the same variant was confirmed.

Clinical data of the three sisters and the father is summarized on [Table 1].

DISCUSSION

To our knowledge, this is the first time that the pathogenic missense variant c.3995G>A in *SCN1A* gene located in exon 20 (NM_001165963, p.Gly1332Glu) is associated with familial "Genetic Epilepsy with Febrile Seizures Plus" (GEFS+)/ DS, although it has been previously linked to *de novo* DS (5). Interestingly, the father had high cognitive abilities, whereas the three daughters had moderate to severe ID. We were only able to confirm the mutation in the father, but we could not rule out that family members with behavioural problems were also carriers of the mutation, but with a milder phenotypic expression. This variable spectrum of the disease is frequent in *SCN1A* missense pathogenic variants (8), since neurodevelopmental impairment seems to be the result of both the underlying genetic variant and the epilepsy severity (9). The burden of EA (especially in early stages of development) could be determinant for cognitive prognosis (10), such in this family, in which subjects with fewer seizures had less disability. Genetic diagnosis was confirmed in the adulthood in the three sisters, after reconsidering the previous diagnosis of LGS (11). This is not unusual, since if not diagnosed at the first instance, the majority of adults (83%) were diagnosed after 4 or more years (12). This fact points out the importance of a careful review of the old medical records, a thorough anamnesis, and a physical exam analysing other neurological signs. Although DS research have been focused in children (13), a correct diagnosis in adults is essential for understating the natural history of the disease and planning treatment (14). It's important to emphasize that a comprehensive and personalized therapeutic approach, incorporating measures such as avoiding the use of sodium channel blockers (SCB) and implementing rehabilitation programs, may potentially assist in enhancing functional outcomes in DS.

The same variant was confirmed postmortem in the father thanks to preserved cerebral tissue. Apart from the pathological hallmarks of AD and common co-pathology, no specific morphological changes related to epilepsy were detected. This is consistent with (otherwise sparse) previous neuropathological DS studies (seven paediatric and one adult), showing non-specific results (1, 2, 3, 4). While AD may indeed obscure some of the previously reported DS findings (such as edema, acute ischemic changes, hyperconvoluted cornu Ammonis and multifocal micronodular dysplasia (1, 2, 3, 4)), some of these findings (such as generalized cortical atrophy, neuronal loss, gliosis, and chronic ischemic changes) could still be observed. Therefore, we believe that there is a variability of neuropathological findings in DS, with some adult cases exhibiting less severe neuropathological features.

Also, brain MRI did not show specific findings, according to previous studies (1, 15), with abnormal MRIs found more frequently in patients without *SCN1A* pathogenic variants (16). Overall, this provides more evidence confirming that the hyperexcitability underlying DS has a functional rather than structural basis.

Additionally, EEGs in surviving sisters did not demonstrate EA, as they are currently seizure-free even after febrile episodes. Adults with DS tend to have a notable reduction in seizures and status epilepticus, but parkinsonian features may be present and progress (13). This heterogeneous expression illustrates the wide expression of Nav 1.1 channel beyond the cerebral cortex (8). We hypothesize that AD could be more frequent in DS, but considering DS patients´ early mortality and poor cognitive outcome, it would be challenging to detect AD during adulthood. Interestingly, a reduction in tau prevented the development of DS in rodents (17). In turn, the presence of EA favoured Aβ deposition (18), and reduced CSF Aβ1-42 levels are more frequently found in individuals with late-onset epilepsy (19). Another interesting finding is the higher mid-life Aβ deposition measured by PET in individuals with childhood-onset epilepsy (20).

CONCLUSIONS

It is crucial to review the clinical presentation and consider carrying out updated genetic studies when facing adult patients with epilepsy and ID, since the diagnosis of entities such as DS may involve important prognosis considerations. The consequences of this

potentially devastating disease seem to have a functional rather than a structural basis, supported by the lack of specific neuropathological findings.

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