Chorea-acanthocytosis: 3 New Families with Novel Genetic and Metabolic Findings

Sir,

Neuroacanthocytosis syndromes are a group of rare heterogeneous neurological disorders characterized by the presence of acanthocytes in peripheral blood smear and basal ganglia degeneration.[1] Chorea-acanthocytosis (ChAc), one of the core neuroacanthocytosis syndromes, is inherited by autosomal recessive transmission and is caused by mutations of the Vacuolar Protein Sorting 13 homolog A (*VPS13A*) gene on chromosome 9q21, which encodes for a protein called "chorein".[2,3]

In this case series of four genetically diagnosed patients from three families of various ethnic origins, we describe two novel mutations of *VPS13A* and highlight elevation of plasma ammonia and lactate as possible disease markers.

Subject 1

A 32-year-old man from South India, born of second-degree consanguineous parentage, presented with history of poorly controlled generalised seizures (both complex partial and generalised tonic clonic) for 10 years. He was tried on multiple anti-epileptic medications (phenytoin, levetiracetam, carbamazepine and lacosamide). He presented to us 10 years later with abnormal orofacial movements for the past 2-3 years. On examination, his cognition and extra-ocular movements were normal. He had orofacial dyskinesia that was more prominent while eating than at rest, along with tongue dystonia. The rest of his neurological examination was normal, except for absent ankle jerks. His laboratory

investigations were normal with the exception of elevated creatinine phosphokinase [CPK] and ammonia [Table 1 Supplementary Figure 1]. In light of the presence of orofacial chorea, he was evaluated for ChAc [Video segment 1]. Genetic analysis by next generation sequencing (NGS) (Agilent Sure Select V5 exome) revealed a novel homozygous missense mutation (Met3088Arg) in exon 69 of *VPS13A* gene (NM_033305.2:c.9263T>G; GRCh38.p13). Here "exon 69" is "exon 70" in the notation of previous work and this transcript (NM_033305.3) contains exons 1–68 and 70–73 of the 73 exons of the *VPS13A* gene.[2] This amino acid substitution of arginine for methionine was predicted to be damaging by the *in silico* prediction tools Polyphen2, SIFT, LRT, and MutationTaster2. This rare variant was absent in population databases [Exome Aggregation Consortium {ExAC}, The Genome Aggregation Database {gnome AD}, 1000 genome], and in ethnically matched individuals [Med Genome database {n = 19,300}]. Sanger sequencing further validated the variant [Figure 1]. This rare clinically correlating variant at the evolutionarily conserved codon was classified according American College of Medical Genetics and Genomics guidelines (ACMG) as variant of uncertain significance due to the lack of sufficient functional evidence/validation.

Subject 2

A twenty-nine year old man born of non-consanguineous parentage from North India presented with an 8-year history of insidious onset of progressive orofacial movements associated with tongue and cheek biting, worse while eating. He used a piece of cloth in his mouth to prevent injury [Video segment 2]. He was previously evaluated for Wilson's disease with copper studies, which were normal. On evaluation, he had orofacial chorea with feeding dystonia. The rest of his neurological evaluation was normal. He was also noted to have elevated ammonia with normal liver enzymes [Table 1]. Genetic analysis revealed a novel homozygous frameshift mutation in exon 34 of VPS13A gene (NM_033305.2: c.3904delG; GRCh38. p13) [Figures 1 and 2]. This mutation is predicted to result in premature truncation of the peptide at 11 amino acids downstream to codon 1302 (p.Glu1302Serfs*11) resulting in the loss of the VPS13A protein C-terminal domains. *In silico* prediction of the variant by the MutationTaster2 tool indicated that it would be damaging, and it was not reported in the population databases including GnomAD consisting of 71,702 genomes from unrelated individuals. The observed loss of function novel variant was classified as pathogenic based on ACMG guidelines. Mutations mostly leading to the absence of the protein have been implicated in the pathogenesis of ChAc.

Subject 3

A 27-year-old woman from eastern India, born of non-consanguineous parentage, presented with a 4-year history of anxiety and depression, for which she was under psychiatric care. She later developed generalized abnormal movements and gait disturbances over next 2-3 years. On examination, her cognition was normal, ocular movements were full, vertical saccades were absent. She had orofacial dyskinesia with tongue dystonia, prominent while eating (feeding dystonia). There was a generalized chorea, multifocal motor tics and a bizarre gait pattern with a combination of axial spasms and chorea ("rubber person" gait). Deep tendon reflexes were absent throughout. The sensorimotor and cerebellar examination was otherwise normal. Her investigations are summarized in Table 1. In view of the clinical pattern and positive peripheral blood smear, she underwent whole exome sequence analysis which identified a previously reported homozygous nonsense variation (Lys1037Ter) in exon 29 of the *VPS13A* gene (NM_033305.3:c. 3109A >T; GRCh38. p13). The substitution results in an early stop codon leading to premature truncation of mRNA translation at codon 1037.[4] The variant was classified as pathogenic based on ACMG guidelines.

Her 25-year-old younger brother, who carried the same mutation, had a two-year history of declining academic

Table 1: Laboratory investigations panels

Investigations	Subject 1	Subject 2	Subject 3
Serum CPK (IU/L) (<145 IU/L)	1600	Not available	316
Serum ammonia (normal range: 11–51/micromole/L, Venous Samples)	124 micromol /L	69 micromol / L	46 micromol/L
Serum lactate	2 mmol/L (0.5-1)	Not available	3.35 mmol/L (0.5-1)
Acanthocytes (Saline dilution method)	8%	10%	7%
EEG	Normal	Not done	Not done
Liver function test	Normal	Normal	Normal
MRI Brain	Normal (age 30); bilateral caudate atrophy (age 32)	Normal	Mild bilateral caudate atrophy
Nerve conduction studies	Mild sensory axonal neuropathy	Not done	Normal
Others	Muscle biopsy-normal	Serum copper and ceruloplasmin-normal	Lipid profile-Normal

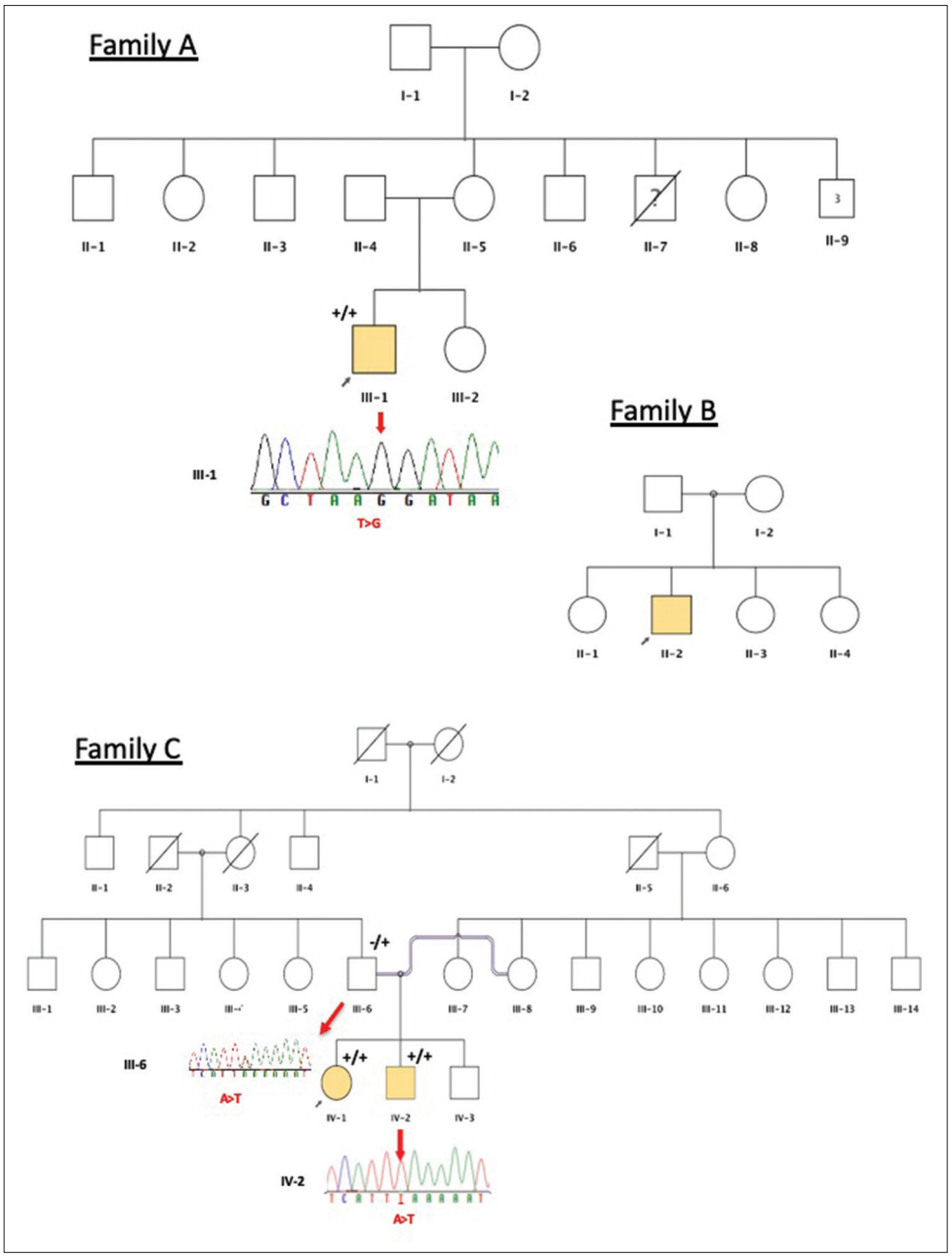

Figure 1: Schematic representation of the partial pedigrees of the three families: circles indicate female and squares indicate male, filled-in squares indicate affected; arrow indicates the consultand. The representative Sanger sequencing chromatogram of the homozygous missense variant (chr9: 80018225T >G; p.Met3088Arg; NM_033305.2) detected in the proband of Family A (III-1) represented as "+" for variant/mutant allele; The representative Sanger sequencing chromatogram of the nonsense variant detected in Family C (chr9: 79897181A >T, p. Lys1037Ter; NM_033305.2). The homozygous variant in the affected is represented as "+" for variant/mutant allele, and the carrier father with one mutant allele "+" and "-" as reference allele

performance, behavioural issues including obsessive compulsive issues. On his initial examination, cognition, eye movements and saccades were normal. There were multifocal motor and vocal tics. Over last two years he developed generalised chorea, bruxism, and intermittent neck flexion episodes.

Our patients presented with the typical, yet variable, clinical symptoms of ChAc, with seizures, psychiatric symptoms, and hyperkinetic movement disorders. Seizures are observed in around half of patients and can be a presenting feature. The isolated presentation with seizures/psychiatric symptoms can often lead to a diagnostic delay, as noted in our first patient.[5-7] Movement disorders are observed in around two-thirds of patients at initial presentation; hyperkinetic disorders (chorea, dystonia, tics, myoclonus) often appear at an early stage, while Parkinsonism occurs in more advanced disease.[1] As this is a very rare disorder, low clinical suspicion

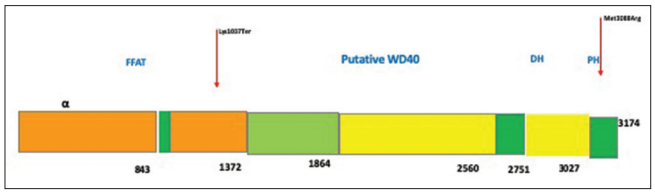

Figure 2: Schematic representation of the domains of VPS13A adapted from Kumar *et al*., 2018.[10] The N-terminal region of unknown fold (VPS13α) consists of the FFAT motif is followed by putative WD40 modules, a domain reminiscent of a DH domain (DH-Like domain; DHL) and a C-terminal pleckstrin homology (PH) domain. The variants detected in this study are indicated by arrow

can result in significant diagnostic delay, as was observed in our second subject.

The persistent CPK elevation during the inter-ictal period can suggest the diagnosis, but prior to the development of a movement disorder, may suggest a mitochondrial or other neuromuscular disorder, potentially leading to an unwarranted muscle biopsy.[5-8] Acanthocytes can be positive in 5–50% patients,[1] and if present, can narrow the diagnosis. Western blot indicating the absence of chorein is supportive in diagnosis.[9] Genetic analysis is considered as gold standard and should be undertaken in presence of strong clinical suspicion.[1]

VPS13A is localised on chromosome 9q21 and has 73 exons. The transcript variant A (exons 1-68, 70-73) is the main expressed form and encodes a 3174 amino acid protein "chorein". The absence/or alteration of this protein results in disease.[1] There are around 84 pathogenic and 9 likely pathogenic variants described so far.[10] These mutations are dispersed throughout the gene without any particular hotspot.[1]

The mechanism by which absent chorein results in basal ganglia neurodegeneration, and the cause of acanthocytes, is not known at present.[1] Chorein is expressed in several cellular organelles and has diverse functions. It is a membrane protein, localised at membrane contact sites including nucleus-vacuole, endoplasmic reticulum (ER)-vacuole and endosome-mitochondria contact sites. It is involved in the transport of membrane bound proteins between trans-Golgi network and prevacuolar compartment and from endosome to vacuole. VPS13A gene mutations can result in impaired autophagic degradation, defective protein homeostasis, delayed endocytic and phagocytic processing, and abnormal calcium homeostasis. The actin polymerization defects, altered protein phosphorylation and red cell membrane properties are postulated to result in acanthocyte formation. Impairment of mitochondrial functions such as fusion and mitophagy could predispose to neurodegeneration. Gene mutations also affect lipid droplet mobility within the cell organelles, leading to their accumulation within neurons and glia, and thus possibly causing neurodegeneration.[11-13]

Two novel mutations were observed in our study. One subject presented initially with seizures, while movement disorders were observed in the second patient. The literature does not support a phenotype-genotype correlation, and significant heterogeneity has been reported within families.[1,5] However, similar to our first subject, there are case reports of patient with seizures as a prominent feature having certain mutations such as c2343 deletions and truncating mutation c.4326 T > A (p. Tyr1442*) in VPS13A.[6,7] Further research on possible phenotype-genotype relations may enhance our understanding of the disease.

We observed elevated fasting serum ammonia levels (in 2/3 of cases; venous sample), which have not been previously reported, nor systematically studied. These subjects had a normal liver function and secondary factors like hepatoxic drugs were absent This assumes significance as hepatic clearance play a major role in metabolism of these substances through the urea cycle and gluconeogenesis, respectively. Patients with ChAc often have hepatomegaly and altered liver function, although this is not usually clinically significant.[1] In addition, lactate was elevated in the two patients in whom it was measured. Muscle biopsy results are either normal or show non-specific neurogenic and myopathic atrophy,[1] while ischemic lactate test[7] and mitochondrial enzymes in muscle were normal.[5-7] The other source of excess lactate could be increased haemolysis, which has been observed in ChAc patients.[1] These findings require further validation in a large number of patients, and may have the potential to serve as an additional disease biomarkers.

In conclusion, ChAc is a rare disorder with varied clinical presentations. The rarity of the condition and low clinical suspicion can lead to a diagnostic delay. Elevation of serum ammonia and lactate levels in the absence of overt liver and muscle disease is intriguing and requires further validation.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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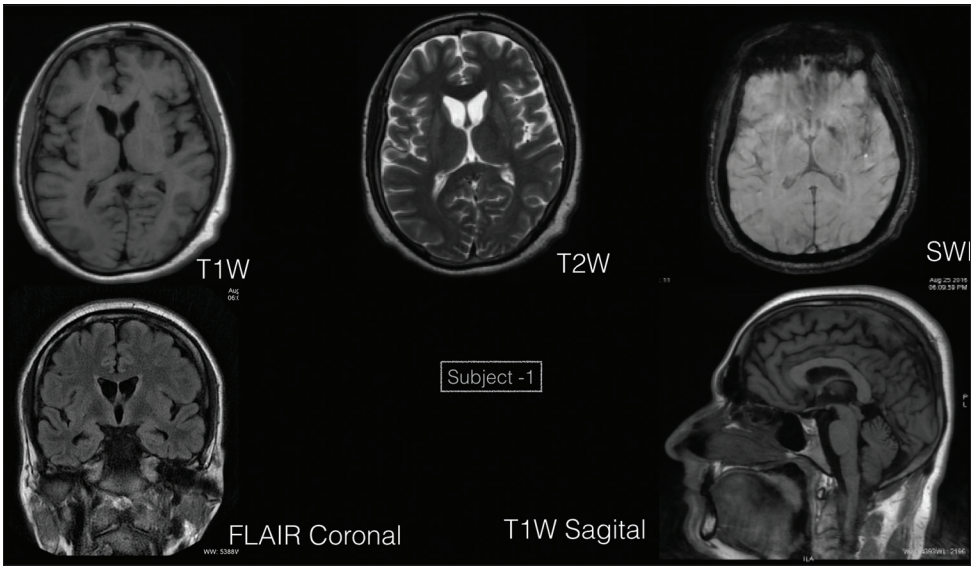

Supplementary Figure 1: Magnetic resonance imaging (MRI) images of subject-1 showing mild diffuse atrophy with along with caudate atrophy atrophy