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Clinical and Genetic Characterization of Autosomal Recessive Spinocerebellar Ataxia Type 16 (SCAR16) in Taiwan

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Abstract

Mutations in *STUB1* have been identified to cause autosomal recessive spinocerebellar ataxia type 16 (SCAR16), also named as Gordon Holmes syndrome, which is characterized by cerebellar ataxia, cognitive decline, and hypogonadism. Additionally, several heterozygous mutations in *STUB1* have recently been described as a cause of autosomal dominant spinocerebellar ataxia type 48. *STUB1* encodes C-terminus of HSC70-interacting protein (CHIP), which functions as an E3 ubiquitin ligase and co-chaperone and has been implicated in several neurodegenerative diseases. In this study, we identified two SCAR16 pedigrees from 512 Taiwanese families with cerebellar ataxia. Two compound heterozygous mutations in *STUB1*, c.[433A>C];[721C>T] (p.[K145Q];[R241W]) and c.[433A>C];[694T>G] (p.[K145Q];[C232G]), were found in each SCAR16 family by Sanger sequencing, respectively. Among them, *STUB1* p.R241W and p.C232G were novel mutations. SCAR16 seems to be an uncommon ataxic syndrome, accounting for 0.4% (2/512) of our cohort with cerebellar ataxia. Clinically, the three patients from the two SCAR16 families presented with cerebellar ataxia alone or in combination with cognitive impairment. The brain MRIs showed a marked cerebellar atrophy of the patients. In conclusion, SCAR16 is an important but often neglected diagnosis of cerebellar ataxia of unknown cause, and the isolated cerebellar ataxia without involvement of other systems cannot be a basis to exclude the possibility of *STUB1*-related disease.

Keywords *STUB1* · CHIP · SCAR16 · Gordon Holmes syndrome · Cerebellar ataxia

Introduction

Hereditary cerebellar ataxias are a clinically and genetically heterogeneous group of cerebellar disorders characterized by slowly progressive gait unsteadiness usually associated with poor coordination of hands, speech, and eye movements.

Mutations in the STIP1 homology and U-Box containing protein 1 (*STUB1*) gene have been originally identified to cause autosomal recessive spinocerebellar ataxia type 16 (SCAR16), also named as Gordon Holmes syndrome, which usually manifests cerebellar ataxia, hypogonadotropic hypogonadism, and cognitive dysfunction [1, 2]. However, a

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wider clinical spectrum of recessive *STUB1* mutations was demonstrated later, presenting as cerebellar ataxia in combination with a variable degree of dementia, spastic tetraparesis, epilepsy, autonomic dysfunction, extrapyramidal symptoms, and hypogonadism [3–6]. Furthermore, heterozygous mutations in *STUB1* have recently been described as a cause of autosomal dominant spinocerebellar ataxia (SCA) type 48 (SCA48) with a later disease onset and similar clinical manifestations to SCAR16 [7, 8].

The *STUB1* gene encodes the C-terminus of HSC70-interacting protein (CHIP), which is a 35-kDa protein and contains three domains, including an N-terminal three tetratricopeptide repeat (TPR) domain, a highly charged middle coiled coil domain, and a carboxyl-terminal U-box domain [2, 9–11]. CHIP functions as both an E3 ubiquitin ligase and a molecular cochaperone [9–11]. Its TPR domain mediates interactions with heat shock proteins, the coiled coil domain influencing dimerization of CHIP, while the U-box domain of CHIP serves as an ubiquitin ligase [9–11]. CHIP facilitates ubiquitylation and maintains protein homeostasis by controlling chaperone levels during stress and recovery [12]. Loss of ubiquitin ligase activity of CHIP has been demonstrated as the molecular mechanism for SCAR16 [2].

Currently, studies about *STUB1* mutations in Han Chinese populations remain sparse, and *STUB1* mutations have rarely been screened in large inherited cerebellar ataxia cohorts before. The aim of this study is to investigate the frequency, clinical manifestations, and spectrum of *STUB1* mutations in a Taiwanese cohort of 512 pedigrees with cerebellar ataxia.

Methods

Patients

All participants were recruited from the Neurology service of Taipei Veterans General Hospital. Mutations in *STUB1* were screened in 108 molecularly unassigned index patients from 512 pedigrees with cerebellar ataxia, after mutations responsible for SCA1, 2, 3, 6, 7, 8, 10, 12, 17, 22, 23, 26, 27, 28, 35, and 36, and DRPLA had been excluded. All participants were of Han Chinese descent. Genetic diagnosis of SCAR16 was made in two index patients by identifying biallelic *STUB1* mutations. Nine individuals from the two Taiwanese SCAR16 families were enrolled into the study, including 3 patients, 5 individuals carrying a single mutant *STUB1* allele, and one with wild type *STUB1* alleles only (Fig. 1a). The patients were thoroughly evaluated by neurological examinations. Age at onset (AO) was defined as the age when the earliest symptoms of truncal or appendicular ataxia occurred according to the statements given by the patients. The clinical severity of ataxia was evaluated using the 40-point (0 being normal) validated Scale for the Assessment and Rating of Ataxia (SARA) [13, 14]. The presence and severity of non-ataxia signs was determined by the Inventory of Non-Ataxia Signs (INAS) [15]. The cognitive function was assessed with mini-mental screening exam (MMSE) [16] and cognitive ability screening inventory (CASI), Chinese version [17]. Brain magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) were performed with a 1.5-T system (Signa EXCITE, GE Medical Systems, Milwaukee, WI). Nerve conduction studies (NCS) and electromyography (EMG) were also performed. Written informed consent was obtained from the participants and the study was approved by the Institutional Review Board of Taipei Veterans General Hospital.

Mutation Analysis

Genomic DNA was extracted from peripheral blood white cells. The exons and their flanking regions of *STUB1* were analyzed by PCR amplification and Sanger sequencing with the intronic primers. The Sanger sequencing was performed by using the Big Dye 3.1 dideoxy terminator method (Applied Biosystems, Foster City, CA, USA) on an ABI Prism 3700 Genetic Analyzer (Applied Biosystems). Amplicon sequences were compared with the reference *STUB1* coding sequence (NM_005861.4). The sequence variations were confirmed by sequencing both sense and antisense strands of the amplicons. The pathogenicity of the novel *STUB1* variants were evaluated by in silico prediction of the functional effects by three bioinformatics tools, Mutation Taster (http://www.mutationtaster.org) [18], Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) [19], and Combined Annotation Dependent Depletion (CADD) (https://cadd.gs.washington.edu/) [20]. Given that *STUB1*-related cerebellar ataxia is a rare disease, the pathogenic variants in *STUB1* should be absent or very rare in the general population. Hence, we surveyed the allele frequencies of the novel *STUB1* variants in the Genome Aggregation Database (gnomAD, v2.1.1) (https://gnomad.broadinstitute.org) and Taiwan biobank (https://taiwanview.twbiobank.org.tw/index) which contains genome data of 1517 healthy Taiwanese individuals. Evolutionary conservation of the mutation sites was analyzed by aligning amino acid sequences of human CHIP and its orthologues utilizing the UniProt website (http://www.uniprot.org) [21].

Results

Genetic Analysis

Mutational analysis of *STUB1* in the 108 index patients with cerebellar ataxia revealed two compound heterozygous variants in *STUB1*, of which c.[433A>C];[721C>T]
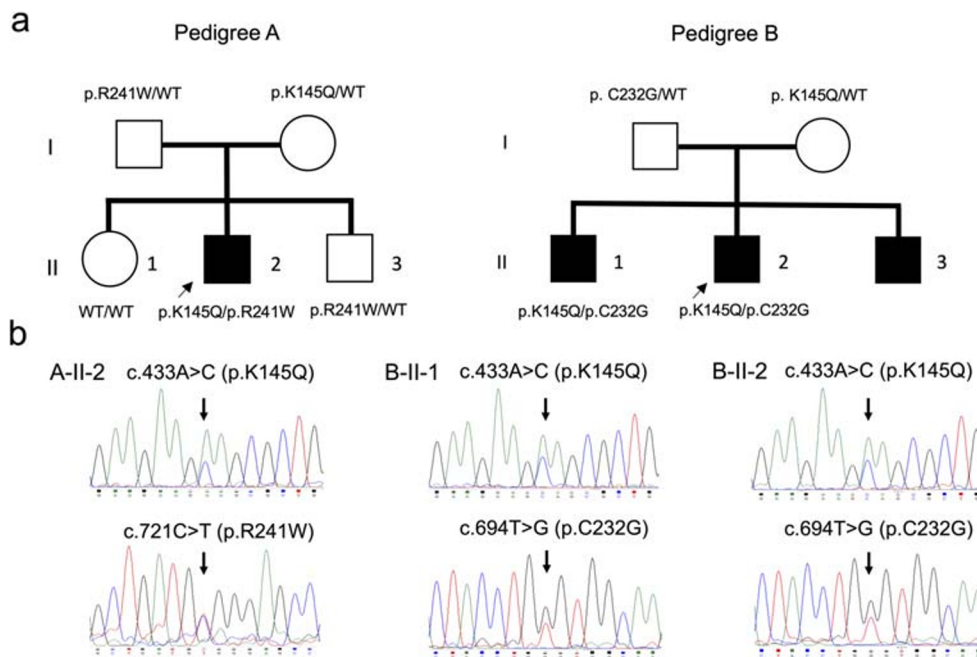
Fig. 1 Pedigrees and electropherograms of the *STUB1* mutations, **a** Pedigree structures of the two families with autosomal recessive spinocerebellar ataxia type 16 (SCAR16). The probands are denoted by an arrow. The squares and circles denote males and females. Filled symbols represent affected members with cerebellar ataxia, and open symbols indicate unaffected individuals. The *STUB1* genotypes are labeled for each individual and "WT" stands for the wildtype allele. **b** The electropherograms of the compound heterozygous *STUB1* mutations identified in the three affected individuals; c.[433A>C];[721C>T] (p.[K145Q];[R241W]) for A-II-2 and c.[433A>C];[694T>G] (p.[K145Q];[C232G]) for B-II-1 and B-II-2

(p.[K145Q];[R241W]) was identified in patient A-II-2 and c.[433A>C];[694T>G] (p.[K145Q];[C232G]) was found in patients B-II-2 (Fig. 1b). The parents of the patient A-II-2 and patients B-II-2 all carried only one single heterozygous *STUB1* mutation. The *STUB1* p.C232G and p.R241W were novel.variants, while the p.K145Q had been well demonstrated as a pathogenic mutation in previous studies [5, 22, 23].

To evaluating the pathogenicity of *STUB1* p.C232G and p.R241W, we utilized three bioinformatics programs. Mutation Taster and polyphen-2 predicted the two *STUB1* variants as disease causing and probably damaging. The CADD v1.4 PHRED scores were 26.7 and 26 for the p.C232G and p.R241W variants, suggesting that they were in the top 0.25% of most deleterious variants in the genome [20]. Both *STUB1* p.C232G and p.R241W were not found in the 1517 ethnically matched control genomes in the Taiwan Biobank database. The p.C232G was absent, and the p.R241W was present with an allele frequency of 0.000799% (2/250190) in the gnomAD, which contained exome or genome data of more than 140,000 individuals. Both mutations alter the amino acid residues of CHIP which are evolutionarily conserved at least from human to fish (Fig. 2).

Clinical Features

Three patients with ataxia from the two SCAR16 families were investigated (Fig. 1a). The first patient (A-II-2) began

to have an insidious onset of gait unsteadiness and dysarthria at age of 29 years (Fig. 1, family A). Neurological examination at age 30 revealed a moderate degree of dysarthria, mild gait ataxia, and cogwheel pursuits, without focal weakness or sensory deficits. The score of SARA was 10.5, and the INAS count was 0. His parents were unremarkable clinically and on the brain MRI. The second patient (B-II-2) started to have progressive gait unsteadiness since age of 22 years. He had
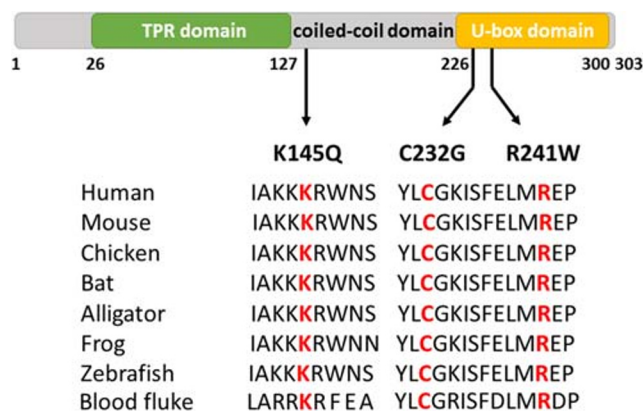

Fig. 2 Schematic representation of CHIP, the protein encoded by *STUB1*, with an N-terminal tetratricopeptide repeat (TPR) domain, a highly charged middle coiled coil domain, and a carboxyl-terminal U-box domain. The *STUB1* p.K145Q, p.C232G, and R241W mutations all alter an evolutionarily conserved amino acid residue, as shown by aligning the amino acid sequences of CHIP orthologues from various species

had one motorcycle accident at age 18 resulting in a head concussion and multiple fractures, with no subsequent sequela or functional impairment. On neurological exam at age 25, he was found to have gait ataxia, dysarthria and cogwheel pursuits. His score of SARA was 21, and the INAS count was 1 for mild cognitive dysfunction. His elder brother (B-II-1) had also experienced dysarthria and gait unsteadiness since age of 37 years (Fig. 1, family B). Their parents had been doing well with normal imaging features on the brain CT. Their younger brother, allegedly also being afflicted with some gait difficulties, was unwilling to be evaluated.

Neuroimaging and Other Laboratory Evaluations

The brain MRI demonstrated a marked cerebellar atrophy without other visible abnormalities (Fig. 3). The MR spectroscopy of brain with the volume of interest located in the cerebellar hemispheres and cerebellar vermis revealed decreased ratios of NAA/Cr in all three patients. Cognitive studies revealed that the cognition was normal in patient A-II-2 (MMSE 30/30; CASI 93/100) but was impaired in patient B-II-2 (MMSE 26/30; CASI 71/100) with lower scores in short-term memory (7/12), abstract thinking (8/12), drawing (6/10), attention (5/8), and verbal fluency (5/10) in CASI. No evidence of hypogonadism was found in the three patients

Fig. 3 Features of T1-weighted brain MRI in patients with autosomal recessive spinocerebellar ataxia type 16 (SCAR16), At examination, patients A-II-2 was a 30-year-old man with disease duration of 1 year, patient B-II-1 was a 39 year-old man with disease duration of 2 years, and patient B-II-2 was a 25-year-old man with disease duration of 3 years

with normal levels of serum follicle–stimulating hormone (FSH), luteinizing hormone (LH), and testosterone. The NCS and EMG showed normal pattern. The tilting table test evaluating the autonomic nervous system function in the three subjects revealed no postural hypotension or postural tachycardia.

Discussion

To understand the features and contribution of *STUB1* mutations in hereditary cerebellar ataxia in our population, we screened 108 unrelated Taiwanese patients with cerebellar ataxia for mutations in the *STUB1* gene and identified two compound heterozygous mutations, p.[K145Q];[R241W] and p.[K145Q];[C232G], in two patients, respectively. No simple heterozygous mutation in *STUB1* was identified. Since the 108 patients were selected from 512 unrelated patients with hereditary cerebellar ataxia after excluding known causative mutations, SCAR16 accounted for 0.4% (2/512) of the Taiwanese cohort with cerebellar ataxia and SCA48 should be absent or very rare in our population. Among the *STUB1* mutations, the p.C232G and p.R241W were novel. Their pathogenicity was supported by the following findings. Firstly, the two mutations alter the evolutionarily conserved amino acid of the U-box domain of CHIP and may compromise its ubiquitin ligase function. Secondly, the *STUB1* p.R241G mutation, which affects the same amino acid as the p.R241W mutation, has been reported in a patient with SCAR16 before [22]. Then, they were absent in the 1517 ethnically matched control genomes in the Taiwan biobank database and absent or present with an extremely rare allele frequency (2/250190) in the gnomAD. Furthermore, either of the two mutations are present in trans with the known pathogenic mutation, p.K154Q, in the patients. Moreover, the p.C232G and p.R241W mutations are both predicted to be pathogenic by Mutation Taster, Polyphen-2, and CADD programs. Based on these findings, the *STUB1* p.c232G and p.R241W mutations may be classified as likely pathogenic variants, according to the guidelines by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology [24].

The clinical presentations and age of disease onset may vary among patients with SCAR16. Autosomal recessive cerebellar ataxia is usually associated with involvement of multiple neurological systems, and SCAR16 has been reported to manifest cerebellar ataxia with a variable combination of hypogonadotropic hypogonadism, cognitive dysfunction, spastic quadriparesis, epilepsy, autonomic dysfunction, and extrapyramidal symptoms [3–6]. However, in our study, the SCAR16 patients presented isolated cerebellar ataxia or in combination with cognitive impairment only. The age of disease onset of SCAR16 in the previous studies is usually before
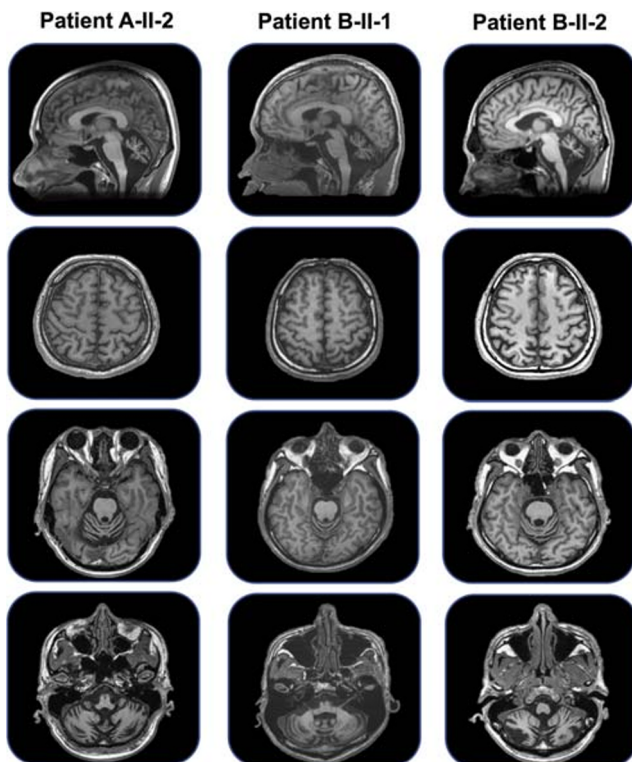
20 years [1–5], while the onset ages of the patients in the present study range from 22 to 37 years. Hence, SCAR16 may manifest adult-onset isolated cerebellar ataxia without involvement of other systems. In addition to the SCAs belonged to the group of autosomal dominant cerebellar ataxia (ADCA) type III [25], such as SCA6, the differential list of isolated cerebellar ataxia should be expanded to incorporate SCAR16.

Since mutations sharing similar features may have similar effects on the encoded protein, it is interesting to correlate the phenotype and genotype of *STUB1* mutations in SCAR16. One study analyzed the clinical features, *STUB1* mutations, and changes of CHIP function in 24 SCAR16 patients and found that *STUB1* mutations affecting the U-box domain of CHIP were linked to cognitive dysfunction [26]. In the present study, only one of the two SCAR16 patients with clear clinical evaluation had cognitive impairment; however, both had one mutant allele altering the U-box domain and the same second mutation, p.K145Q, located in the middle coiled coil domain of CHIP. The discrepancy of the phenotypic and mutational features between these two patients may come from many factors. CHIP does not exert its function alone, and any genetic or environmental factor influencing the functional pathways which CHIP participates in may modify the SCAR16 phenotype. Actually, intra-familial phenotypic variation may exist in SCAR16 and present with variation in age of ataxia onset as well as presence or absence of cognitive dysfunction [1, 5]. In the other hand, SCAR16 is a rare disease, and only a small number of SCAR16 cases with variable homozygous or compound heterozygous *STUB1* mutations had been reported, so it may be premature to conclude whether there is a clear genotype–phenotype relationship in SCAR16.

One limitation of the present study is that we might underdiagnose the SCAR16 cases in our ataxia cohort because of limitation of the methodology. We did not investigate large segment deletions of total or a part of the *STUB1* gene in this cohort. Since the molecular mechanism of SCAR16 has been demonstrated as loss of ubiquitin ligase activity, large segment deletion affecting partial or entire *STUB*1 may result in loss of CHIP function and cause SCAR16. However, we did not find any ataxia patient carrying a single *STUB1* mutation by Sanger sequencing in our cohort, and biallelic large segment deletion of *STUB1* should be extremely rare. Therefore, this issue might not influence our study substantially.

Conclusion

This study expands the clinical and mutational spectrum of SCAR16. We identified three SCAR16 patients carrying compound heterozygous mutations in *STUB1* and manifesting an adult-onset cerebellar ataxia alone or in combination with cognitive impairment from a Taiwanese cohort with cerebellar ataxias. *STUB1* p.R241W and p.C232G are novel pathogenic mutations. Mutations in *STUB1* are not a common cause of cerebellar ataxia; however, SCAR16 is still an important but often neglected diagnosis of cerebellar ataxia of unknown cause, and the isolated cerebellar ataxia without involvement of other systems cannot be a basis to exclude the possibility of *STUB1*-associated ataxia.

Compliance with Ethical Standards

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