Clinical and genetic spectrum of a Chinese cohort with *SCN4A* gene mutations

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Abstract Skeletal muscle sodium channelopathies due to *SCN4A* gene mutations have a broad clinical spectrum. However, each phenotype has been reported in few cases of Chinese origin. We present detailed phenotype and genotype data from a cohort of 40 cases with *SCN4A* gene mutations seen in neuromuscular diagnostic service in Huashan hospital, Fudan University. Cases were referred from 6 independent provinces from 2010 to 2018. A questionnaire covering demographics, precipitating factors, episodes of paralysis and myotonia was designed to collect the clinical information. Electrodiagnostic studies and muscle MRI were retrospectively analyzed. The clinical spectrum of patients included: 6 Hyperkalemic periodic paralysis (15%), 18 Hypokalemic periodic paralysis (45%), 7 sodium channel myotonia (17.5%), 4 paramyotonia congenita (10%) and 5 heterozygous asymptomatic mutation carriers (12.5%). Review of clinical information highlights a significant delay to diagnosis (median 15 years), reports of pain and myalgia in the majority of patients, male predominance, circadian rhythm and common precipitating factors. Electrodiagnostic studies revealed subclinical myotonic discharges and a positive long exercise test in asymptomatic carriers. Muscle MRI identified edema and fatty infiltration in gastrocnemius and soleus. A total of 13 reported and 2 novel *SCN4A* mutations were identified with most variants distributed in the transmembrane helix S4 to S6, with a hotspot mutation p.Arg675Gln accounting for 32.5% (13/40) of the cohort. Our study revealed a higher proportion of periodic paralysis in *SCN4A*-mutated patients compared

with cohorts from England and the Netherlands. It also highlights the importance of electrodiagnostic studies in diagnosis and segregation studies.

Key Words

skeletal muscle sodium channelopathy; Nav1.4; SCN4A; clinical spectrum; genetic testing; Electrophysiology

Introduction

Skeletal muscle channelopathies are inherited neuromuscular disorders caused by mutations in sarcolemmal ion channel genes. The voltage-gated sodium channel (Nav1.4) coded for by the *SCN4A* gene is essential for the generation and propagation of action potentials [1, 2] playing a crucial role in muscle membrane excitability. *SCN4A*-related skeletal muscle channelopathies have a wide clinical spectrum including autosomal dominant myotonic disorders (Sodium Channel Myotonia, SCM or Paramyotonia Congenita, PMC), exercise- and cold-induced muscle cramps/myalgia[3], the periodic paralyses (Hyperkalaemic Periodic Paralysis, HyperPP, or Hypokalemic Periodic Paralysis , HypoPP), and the autosomal recessive conditions of congenital myasthenic syndrome (CMS), and severe fetal hypokinesia/ congenital myopathy[4]. Different mutations in *SCN4A* result in specific gain-of-function or loss-of-function pathological defects that give rise to the diverse clinical spectrum[5, 6]. To date, over 82 variants have been identified in the *SCN4A* gene and molecular physiological studies have helped in establishing genotype-phenotype correlations. In European populations, SCN4A-related channelopathies have a prevalence of 0.39-0.87/100,000 [7, 8]. However, only a small

number of case series have been reported, in Asian populations [9-14].

In this study, we reviewed the clinical and genetic spectrum, electrophysiology and radiological features of 40 Chinese patients with *SCN4A* gene mutations.

Materials and Methods

Genetic Analysis and Patient Ascertainment

Patients with either periodic paralysis and/ or myotonia were referred to the Department of Neurology, Huashan Hospital from 2010 to 2018 from Jiangsu, Zhejiang, Jiangxi, Anhui, Henan and Hebei Provinces in East, South and North China. *SCN4A* variants were identified by targeted next generation sequencing (NGS) and subsequently confirmed by Sanger sequencing as described in a previous report [11]. Genetic variants were interpreted in the context of the co-segregation analysis and American College of Medical Genetics and Genomics (ACMG) guidelines. Asymptomatic carriers were first-degree relatives verified by co-segregation analysis. The study was approved and performed under the guidelines of the Institutional Ethics Committee of Huashan hospital. Written informed consent was acquired from each participant.

Clinical data collection

We collected clinical data by designing a questionnaire for patients with primary skeletal muscle ion channelopathies. The questionnaire included details of demographic data, presenting symptoms and disease progression, past medication and family history (Suppl.Fig.1). Details of serum potassium and creatine kinase levels during the attacks

were also collected. The questionnaire was completed by patients in the outpatient clinic or via telephone inquiry. The patients were sub-grouped according to clinical manifestations and electrodiagnostics [15, 16]. SCM was characterized by pure myotonia without muscle weakness. PMC was defined as prominent paradoxical myotonia with some symptoms of periodic paralysis. For the cases with periodic paralyses, the timing and duration of attacks, serum potassium levels, dietary triggers and previously characterized genotype-phenotype correlations were taken into consideration for classification.

Electrodiagnostic studies and Muscle Imaging

We performed needle electromyography (EMG) on a total of 11 muscles including axial, upper and lower limb muscles using an EMG device and software (Medtronic, keypoint.net) (Suppl.Tab.1). Myotonic prevalence was defined as the ratio of muscles with myotonic discharges to the total number of tested muscles. Motor nerve conduction velocity (MNCV) measurement was performed in peripheral nerve including median, common peroneal, and tibial nerve. Sensory nerve conduction velocity (SNCV) measurement was performed in median, ulnar, sural, and superficial peroneal nerve. Long exercise test (LET) was performed according to the Fournier protocol[17]. A significant decline (≥30%) of CMAP amplitude compared to baseline value is considered to be abnormal for LET within 45 minutes[18-20]. Short exercise test (SET) was performed followed the protocol set up by Fournier et al with three repetitions [18-20]. MRI at 3T (Philips Ingenia, Holland) was used to scan the patients in a supine position with surface array coils. T1 weighted image (T1WI) and short time inversion recovery (STIR) sequences were employed.

Statistical Analysis

Kruskal-Wallis test and Mann-Whitney test were used to compare the differences among groups because of the abnormally distributed data (Shapiro-Wilk test) and relatively small sample size. Continuous variables were presented as means ± standard deviation (SD) or median (interquartile range, IQR) when appropriate. Categorical variables were expressed as frequencies (%). Statistical significance is set at the 95% level ($P < 0.05$). The data was analyzed using SPSS (version 22).

Result

Clinical characteristics of patients with *SCN4A* gene mutations

A total of 40 cases including 35 patients and 5 asymptomatic carriers of Chinese Han origin from 27 unrelated families were enrolled, including 33 males (31 symptomatic and 2 asymptomatic) and 7 females (4 symptomatic and 3 asymptomatic). The median age of onset in symptomatic patients was 12(range 3, 16.3) years old, while the average diagnostic age was 29(range 22.5, 36.5) years.

Patients were classified into five diagnostic groups: 7 with SCM, 4 with PMC, 6 HyperPP, 18 HypoPP and 5 asymptomatic mutation carriers. The clinical data is listed in Tab.1. The onset age was significantly different among groups (P=0.008), while as for diagnostic age, there was no difference (P=0.417). The average time for all patients from onset to achieving a genetic diagnosis is 15 (11,27) years (Tab.2). A delay to diagnosis was particularly prominent in patients with non-dystrophic myotonia with 26 (20,35) years for

SCM, and 24.5 (11,41.8) for PMC respectively.

Thirty patients (30/35, 85.7%) provided feedback to the questionnaires. The top 3 precipitating factors for all participants were sedentary lifestyle (20/30, 66.7%), cold (19/30,63.3%), and strenuous exercise(15/30,50%). Other precipitating factors included fasting(10/30,33.3%), drinking alcohol (5/30,16.7%), upper respiratory tract infection(4/30,13.3%), satiation(4/30,13.3%), glucocorticoid administration (2/30,6.7%), sleep deprivation(2/30,6.7%), high-carbohydrate intake(2/30,6.7%), diarrhea(1/30,3.3%), and vomiting(1/30,3.3%) (Fig.1). With regard to the accompanying symptoms, 56.7%(17/30) patients experienced muscle pain, which was more prevalent in Hyper/HypoPP groups than in SCM/PMC groups (75% [15/20] VS 20% [2/10]).

We then reviewed the paralytic attacks for 18 patients with periodic paralysis. According to the episodic frequency, we divided them into two subgroups: high frequency group with average attack $\geqslant$1 per month (Fig.2A) and low frequency group with average attack <1 per month (Fig.2B). Patients from high frequency group carried different mutations (p.R675Q, p.R1451L, p.T704M, and p.F1290L), clinically presenting different phenotypes (HypoPP, HyperPP, and SCM). For 83.3% patients (15/18) the most frequent attacks were between the ages of 14 and 30 years old. With regard to the time of day when paralysis occurred, 90% patients (18/20) were often paralyzed when they woke up in the morning or at midnight (Fig.2C).

For patients with SCM/PMC, up to 80% (8/10) cases had frequent myotonia during cold winter months from January to March in China, while only 50% (9/18) Hyper/HypoPP

patients had frequent attacks in winter (Fig.2D).

Clinical Electrophysiological studies

Needle EMG study revealed myotonic discharges in all tested muscles in SCM and PMC patients, with one exception of rectus abdomini in a SCM patient (P4-1) and masseter in a PMC patient (P8-2). The father of a SCM patient (P3-2) reported himself as asymptomatic. This was despite percussion myotonia in both the thenar and tongue muscles and the myotonic discharges identified in all 11 muscles tested. Four HyperPP patients (P9-1, P11-1, P11-2, and P12-1) and 1 homozygous HypoPP (P27-1) patient had myotonic discharges (Suppl.Tab.2). From a clinical perspective, P12-1 reported decreased ability to relax after a forced grip in hands and eyelid closure. P27-1 did not complain of any myotonic symptoms but had evidence of eyelid myotonia on clinical examination.

Electrodiagnostic studies also revealed myogenic changes in 7 patients (1 with SCM, 2 with PMC, 2 with HyperPP and 2 with HypoPP) and neurogenic changes in 1 HypoPP patient (P15-1). Myogenic discharges were more prevalent in lower limbs, particularly in vastus medialis, tibialis anterior and medial gastrocnemius. For Patient 15-1, we identified polymorphic and giant motor unit potentials in flexor carpi radialis, biceps, vastus medialis, medial gastrocnemius and tibialis anterior without any detectable causes like diabetes or neural root injury. The amplitude for both common peroneal nerves was slightly reduced (2.2mV on the left; 2.6mV on the right), while the velocity remained normal. These 8 cases had no significant difference in age of onset (11[2.5,14.75] VS 12[3,17] years old, p=0.791) nor the course of disease (15[11,23.5] VS 15.5[10.75,30.75] years, p=0.776) compared

with other patients.

For the LET results, no SCM cases had significant CMAP amplitude decline, while all PMC, and periodic paralysis subgroups had a CMAP decline > 30% of baseline value within 45 minutes. Two patients (8-1, 8-2) with PMC showed specific SET pattern: the amplitude suddenly decreased 20-50% at 10-20s after the first exercise, then continued to decline. The largest decrement was 74.4% for P8-1 and 63.7% for P8-2. For the asymptomatic carriers of p.R1451L heterozygous mutation, the proband's father (P27-2) and sister (P27-4) had a significant CMAP decline of > 30% of baseline value at 25 minutes and 10 minutes after exercise respectively. Their mother (P27-3) had a slight CMAP amplitude decline but this did not reach 30% of the baseline amplitude.

Muscle MRI in lower limbs

Two patients (P18-1 and P25-1) performed muscle MRI in lower limbs. We did not observe significant abnormalities in thigh muscles. In the calf, we found symmetrical fatty infiltration and muscle atrophy in gastrocnemius for patient 25-1 and left soleus involvement in patient 18-1. Muscle edema of bilateral gastrocnemius was present in both cases (Fig.3).

Nav1.4 mutations identified in the Chinese cohort

A total of 15 mutations were identified in this study, including 13 known mutations and 2 novel mutations (c.G718A p.Val240Met and c.T1862A p.Phe621Tyr). All *SCN4A* mutations were inherited in an autosomal dominant pattern except one patient (P27-1) with HypoPP and myotonia due to a homozygous p.R1451L mutation[11]. Two heterozygous first-degree relatives did have a positive LET consistent with periodic

paralysis despite denying clinical symptoms suggesting an inheritance pattern of autosomal dominant with reduced penetrance cannot be fully excluded in this pedigree. Most mutations were distributed in the transmembrane portion of the helix S4-S6: 4 mutations in S4, 2 mutations in the S4-S5 linker, 2 mutations in S5, 2 mutations in S6 and 2 mutations in the S6-S1 linker (Fig.4). Two novel mutations, c.G718A p.V240M and c.T1862A p.F621Y, were identified in a HypoPP patient(P13-1) and a PMC patient(P6-1) respectively. The missense mutation, p.V240M, was predicted to be probably damaging (Score 1, Polyphen2), or damaging (Score 0, SIFT). Another missense mutation, p.F621Y, was predicted to be possibly damaging (Score 0.741, Polyphen2) and damaging (Score 0, SIFT). Detailed genetic variants and associated phenotypes are listed in Tab.1.

Twelve reported *SCN4A* mutations correlated well with previously characterized clinical subtypes [10-12, 21-26]. A hotspot mutation p.Arg675Gln was revealed with a proportion of 32.5% (13/40) in our cohort. This mutation has been linked to HypoPP in previous molecular electrophysiological studies[2, 27].

Discussion

Our retrospective single center study includes 40 cases with *SCN4A* mutations and represents the largest series reported to date for Chinese population. The phenotypic spectrum ranged from pure myotonia, myotonia and transient weakness overlapping with one or other symptom predominating, or pure periodic paralysis to clinically asymptomatic carriers with a positive long exercise test or EMG evidence of electrical myotonia. With the

application of a disease-relevant questionnaire, we identified a male predominance, high attack frequency in late adolescence to early adulthood, and during early morning and winter months of the year. Our data also highlights a significant delay to diagnosis for this group of disorders in China.

The proportion of the 4 different subtypes in our *SCN4A* mutation cohort differed from previous studies[7, 8]. For the symptomatic cases, our cohort had a large proportion (68.57%, 24/35) of patients with periodic paralysis and the rest 28.57% (10/35) presented with non-dystrophic myotonia, which are significantly different from the studies in England and the Netherlands with a high proportion (57.7%-88.9%) of myotonia and fewer patients with periodic paralysis [7, 8]. Geographical and Ethnic differences, selection bias for a single center study and the relatively small sample size may be responsible for some of the differences. As previously described, the patients with PP had a later age of onset than SCM or PMC patients[2, 16]. However, patients with SCM/PMC had a longer delay to diagnosis compared with the patients with periodic paralysis, which may be contributed to more nebulous clinical complaints of non-specific muscle stiffness and the insufficient use of electrodiagnostic studies. A high prevalence of muscle pain was disclosed from the cohort. Though not specific, myalgic phenotype had been previously reported as the chief complaint in patients p.A1156T sodium channel mutation[3]. Muscle stiffness or pain should be paid more attention in clinical settings to make better diagnosis for milder sodium channel mutations.

Male predominance was particularly evident in our study. Male to female ratio in symptomatic patients was 31 to 4 and 60% of asymptomatic carriers were female. Even

for the same mutation (p.V445M, p.R675Q and p.T704M), female patients had lower attack frequency and milder severity than males. This finding is in line with the observation in previous studies[28, 29]. The underlying mechanism of the gender bias remains to be determined, but sex hormones as natural regulators for different voltage-gated ion channels are suspected to contribute to this gender bias. An in-vitro study with mouse myotube C2C12 cells showed that voltage-gated sodium current density decreased with androgen treatment with unchanged sodium channel mRNA level, indicating that androgen impairs the function of Nav1.4 in a post-transcriptional way[30]. Thus, we hypothesize that hormones affect the function of Nav1.4, which might explain the accelerated paralytic attacks in adolescence and young adulthood.

A circadian rhythm for periodic paralysis with more attacks at night or early in the morning was observed. The seasonal preference in SCM and PMC patients can be ascribed to cold sensitivity, one of the features of non- dystrophic myotonias[16]. Circadian regulation is reported in renal epithelial sodium channels and other neuronal ion channels[31, 32]. However, there are no studies regarding circadian rhythm for skeletal muscle ion channels as yet.

Clinical electrophysiological studies play a vital role in the diagnosis of skeletal muscle sodium channelopathies[17, 18]. Apart from the prevalent myotonia in SCM and PMC patients, we also identified myotonia in an autosomal recessive HypoPP patient, in accordance with the molecular electrophysiology findings that the mutation hastens recovery rate from inactivation[11]. Notably, EMG myotonia and a positive long exercise test was demonstrated in individuals reporting no clinical symptoms e.g., carrier of

p.R1451L, indicating that electrophysiological studies provide a sensitive tool in diagnosing skeletal sodium channelopathies and play a vital role in segregation studies to determine the pathogenicity and inheritance pattern of genetic variants. Further, exercise testing helped distinguish the pure myotonic phenotype of SCM from other phenotypes with myotonia and transient weakness such as PMC and Hyper/HypoPP. Consistent with previous studies, we identified muscle edema and fatty infiltration in posterior compartments in the calves on MRI[33, 34]. However, whether fixed myopathy develops in these muscle would require long-term visits [24, 35, 36].

We identified two novel SCN4A mutations, c.G718A p.V240M in a HypoPP patient, and c.T1862A p.F621Y in a PMC patient. Although they have been predicted to be damaging by online software, the in vitro functional analysis is still required to further assess the pathogenicity of these two variants.

In concordance with the previous reports, we found that HypoPP patients were more likely to have mutations causing neutralization in positively charged amino acids in one of the voltage sensors[6]. Previous studies revealed that HypoPP-related SCN4A mutations were often located in Domain I, II, and III. Here we identified a homozygous mutation in Domain IV voltage sensor region which is related to HypoPP. All hyperPP-related mutations were distributed in S5-6, which are close to the ion selection pores. Other research focusing on HyperPP genotype-phenotype correlation mentioned reduced slow sodium channel inactivation may be the cause for HyperPP[37]. The relation between ion selection pore region and slow sodium channel inactivation needs further investigation.

In conclusion, our report of this Chinese cohort with SCN4A mutations provides detailed information about the phenotype-genotype spectrum. We reveal a higher proportion of periodic paralysis in *SCN4A*-mutated patients compared with cohorts described from England and the Netherlands. Notable findings include a significant delay to diagnosis, frequent reports of pain/myalgia and the utility of EMG and the long exercise test in segregation studies.

Declaration of Interest

The authors declared no conflicts of interest.

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Figure legends

Fig.1. Precipitating factors for Nav1.4 mutation-related channelopathies identified in this study were presented in the order of most to least common occurrence. The top three precipitating factors were sedentary lifestyle (20/30,66.7%), cold (19/30,63.3%) and strenuous exercise (15/30,50%). Sedentary lifestyle: an inactive lifestyle with a lot of sitting or lying down or squatting. URI: upper respiratory tract infection.

Fig.2. Attack frequency and time preference in patients with Nav1.4 mutation-related channelopathies. Patients with periodic paralysis were sub-grouped into high attack frequency group (average attack frequency $\geqslant$1 per month, n=6) (A) and low attack frequency group (1 per year < average attack frequency <1 per month, n=12) (B). All frequencies were normalized as a relative ratio to the individuals' original frequency. Circadian rhythm and seasonal preference for the frequencies of both myotonia and periodic paralysis was analyzed in all Nav1.4 mutation-related channelopathies responsive to the questionnaires (C-D).

Fig.3. The muscle MRI of two patients (P18-1, P25-1) with the same mutation p.R675Q shared similar pattern in lower limbs. Fatty infiltration, muscle atrophy and muscle edema were found symmetrically or asymmetrically in calves, while thigh muscles remained normal. The yellow arrows pointed out the fatty infiltration and edema in bilateral gastrocnemius.

Fig.4. Fifteen mutations were identified in our cohort. The four phenotypes were illustrated by different colored dots. Red, purple, blue and green dots respectively

represented SCM, PMC, NormoPP and HypoPP. Two novel mutations were marked with *.

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