Genomics Data 4 (2015) 65–68

Contents lists available at ScienceDirect

Genomics Data

journal homepage: http://www.journals.elsevier.com/genomics-data/

Identification of genetic variations of a Chinese family with paramyotonia congenita via whole exome sequencing

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ARTICLE INFO

Article history:
Received 6 January 2015
Received in revised form 3 March 2015
Accepted 3 March 2015
Available online 18 March 2015

Keywords:
Paramyotonia congenita (PC)
Neuromuscular disorder
Sodium channel
Voltage-gated type IV alpha subunit (SCN4A)
Whole exome sequencing (WES)

ABSTRACT

Paramyotonia congenita (PC) is a rare autosomal dominant neuromuscular disorder characterized by juvenile onset and development of cold-induced myotonia after repeated activities. The disease is mostly caused by genetic mutations of the sodium channel, voltage-gated, type IV, alpha subunit (SCN4A) gene. This study intended to systematically identify the causative genetic variations of a Chinese Han PC family. Seven members of this PC family, including four patients and three healthy controls, were selected for whole exome sequencing (WES) using the Illumina HiSeq platform. Sequence variations were identified using the SoftGenetics program. The mutation R1448C of SCN4A was found to be the only causative mutation. This study applied WES technology to sequence multiple members of a large PC family and was the first to systematically confirm that the genetic change in SCN4A is the only causative variation in this PC family and the SCN4A mutation is sufficient to lead to PC.

Introduction

Paramyotonia congenita (PC), first described by von Eulenburg, is an autosomal dominant disorder characterized by increased stiffness on repeated activities and cold-induced skeletal muscle myotonia [1]. The onset of PC usually begins early in life, with myotonic discharges as the chief abnormal features of electromyographic recordings [1,2]. In most known cases, PC is caused by a single nucleotide mutation in the SCN4A gene that encodes the alpha-subunit of the skeletal muscle sodium channel [3]. The SCN4A gene is located on chromosome 17q23–25 and consists of 24 exons with a 5.5-kb open reading frame [4,5]. The SCN4A protein comprises 1836 amino acids and mediates the initiation and propagation of action potentials in skeletal muscle cells [4,5]. It contains four homologous domains (DI, DII, DIII, and DIV) and each domain possesses six transmembrane alpha-helical segments (S1, S2, S3, S4, S5 and S6), of which, the S4 segment is thought to be involved in voltage-dependent gating of the skeletal muscle sodium channel, based on its high concentrations (Fig. 1) [5].

With the development of whole exome sequencing (WES), it has now become possible to take advantage of this powerful and cost-effective new tool for analyzing the genetic basis of diseases and features that have proved to be unyielding to conventional gene-discovery strategies [6]. Consequently, the sequence of an individual's entire exome and even genome will quickly become available, enormously facilitating clinical screening and diagnosis [7,8]. In this study, we applied WES to analyze the genetic composition of a family, whose members were affected by PC, and successfully identified the mutation R1448C of the SCN4A gene. Since the disease is very rare, our findings will be a valuable addition to the genetic and epidemiological data of PC in the Chinese population.

Results

Preliminary analysis using NextGENe™ software

Considering that PC is transmitted in an autosomal dominant pattern, we assumed that heterozygous mutations were the underlying cause for the disease the patients were suffering. Through NextGENe™ software processing with the human genome GRCh37/hg19 as the reference, we obtained 2,077,720 to 2,458,338 single nucleotide variations (SNV) and 345,175 to 453,177 InDels (short insertions or deletions) from the four patients and three healthy family members (see details in Table 1).

Further screening via bioinformatic methods and Sanger sequencing

With the removal of the described mutations that were also present in the healthy members, the shared SNVs and InDels were significantly reduced to 14 and 3, respectively. After filtering the remaining mutations against the multiple databases, including the dbSNP, HapMap, the 1000 Genome, and in-house exome data (from the Beijing Genomics Institute, Shenzhen, China), the candidate variants were

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http://dx.doi.org/10.1016/j.gdata.2015.03.002

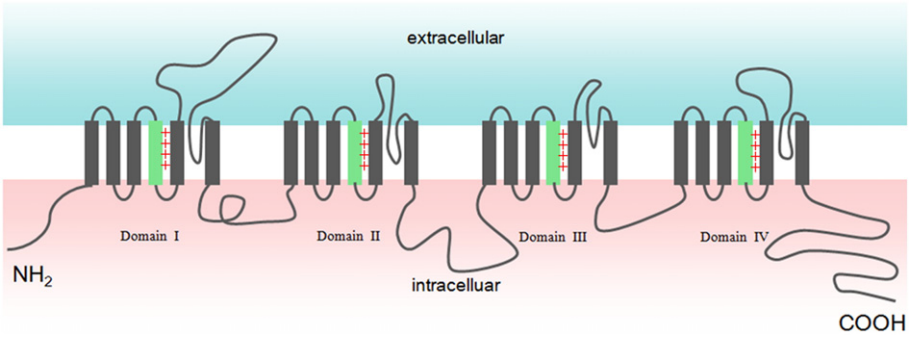

Fig. 1. Voltage-sensitive sodium channel α-subunit. The channel consists of four homologous domains (DI, DII, DIII, and DIV), each with six transmembrane α-helix segments (S1, S2, S3, S4, S5, and S6). In each domain, the S4 segment (green block) is suggested to be involved in voltage-dependent gating of the skeletal muscle sodium channel due to the high concentration of positive charge in S4 segment. The high concentration of positive charge in this α-helical segment suggests that the S4 segment is involved in voltage-dependent gating (red plus symbol).

reduced to 12 SNVs and 3 InDels. The tolerated variants were removed via bioinformatics methods, mainly the SIFT software (http://sift.jcvi.org) [9]. Afterwards, only 6 SNVs and 3 InDels were left. Sanger DNA sequencing was performed with the remaining family members and 5 mutations were also found in the additional healthy members. After sequencing confirmation, 2 SNV and 2 InDels remained to be associated with all the patients (Table 2). As expected, the SCN4A mutation existed in the screened list. The missense mutation (the c.4342G>A [p.Arg1448Cys]) identified in this family was previously reported as the causative variant for PC [10].

Discussion

In our current study, we performed a systematic WES to identify the causative variant leading to PC in a large PC family in China. We found mutations and InDels in four genes associated with PC after sequencing and mutational analysis. To further narrow the gene list, we performed an additional reference search. The gene adenylate cyclase 10 (ADCY10) encoding the soluble adenylyl cyclase plays a critical role in mammalian spermatogenesis [11]. Genetic variations in this gene have been reported to be associated with absorptive hypercalciuria [12]. Research has also shown that variations in ADCY10 may have a modest effect on peak spinal bone mineral density (BMD) in healthy women [13]. Mouse Genome Informatics (MGI) has described a knock-out type ADCY10^{tm1Lex} that replaced exons 2, 3 and 4. Without the main structure of the catalytic domain, the knock-out mouse exhibited a variety of infertility symptoms. As a consequence, mutation c.1162T>C (p.388S>GS) of ADCY10 was unlikely to be the causative gene of the patients' disorder. The neuromedin U (NMU) gene encodes Neuromedin U that stimulates muscle contraction of specific regions of the gastrointestinal tract. In humans, NMU stimulates the contraction of the ileum and urinary bladder [14]. Abnormalities of this gene almost invariably lead to overweight and obesity [15]. With the insertion of c.86_87insAGC, which causes the frameshift, NMU will lose the active catalytic domain. MGI has been shown to produce a knock-out type NMU^{tm1Mko} with PGK-Neo replacing exon 9 (the active C-terminal portion of NMU) via homologous recombination. Obesity represents one of the significant symptoms of the knock-out mice, which also include increased fat cell size, hypoactivity, impaired adaptive thermogenesis and hepatic steatosis. In addition, a large number of studies have demonstrated that mutations in the NMU gene is associated with cancer [16–18]. Therefore, the NMU gene may not have contributed to PC of the patient family.

Gene proprotein convertase subtilisin/kexin type 4 (PCSK4) encodes a member of the subtilisin-like proprotein convertase family and is expressed only in the testis, placenta, and ovary. In rodents, deletion of PCSK4 results in severely impaired fertility in the absence of any evident spermatogenic abnormality [19]. Mutations identified in the collected PC family may represent individual variation, instead of disease association.

After elimination of the abovementioned genes, SCN4A remained as the only possible pathogenic gene with connections to the disease. Previous research has demonstrated that mutations of SCN4A were the causative factors for paramyotonia congenita [5]. Based on the results of our analysis, the variant c.4342G>A (p.1448R>RC) seems to be partially or even solely responsible for the PC disease (Fig. 2A). The mutation in SCN4A, which was further confirmed by Sanger sequencing (Fig. 2B), altered the 1448th codon CGA for arginine into TGA for cysteine. Consequently, this missense mutation resulted in a change in electric charge, which transformed the positive charge into neutral [10]. The gene SCN4A encodes sodium channel, voltage-gated, type IV, alpha subunit (Fig. 1), which plays a primary role in action potential initiation and propagation in excitable cells, including nerve, muscle and neuroendocrine cell types [20]. Structurally, the protein has four homologous domains (DI, DII, DIII, and DIV), each of which consists of six transmembrane alpha-helical segments (S1, S2, S3, S4, S5, and S6). The S4 segment in each domain contains from four to seven repeated three-residue motifs of a positively charged amino acid (usually arginine) followed by two hydrophobic amino acids [21]. The high concentration of positive charges in this alpha-helical segment suggests that the S4 segment is involved in voltage-dependent gating [5].

WES has become increasingly popular and has already shown promise as a valuable diagnostic tool for patients with confusing phenotypes and features suggesting a primary genetic etiology, such as a strong family history, developmental anomalies, or uncustomary presentations of common diseases [22,23]. Through this powerful technology, the PC phenotype-causing variant of this family was identified as the R1448C mutation of gene SCN4A. The R1448C mutation of SCN4A is commonly considered as the causative factor. This variant is consistent with epidemiological data from other countries [10,21,24]. According to previous studies, the most representative features of myotonia may be explained by the abnormal hyperexcitability of skeletal muscle induced by altered inactivation. The mutation R1448C of gene SCN4A slows down the inactivation rate of the skeletal muscle sodium channel and invalidates rapid recovery from inactivation [10]. As a result of the alterations in channel kinetics, there is a prolonged inward (depolarizing) current following muscle excitation, which may lead to myotonia.

Table 1
Summary of the mutation data from seven members of the PC family.

Sample ID	Mismatches count.	InDels count.
Patient I-2	2135048	388705
Patient II-1	2148577	348610
Patient II-9	2185421	345175
Patient III-1	2200776	404206
Healthy member III-3	2457514	453177
Healthy member III-4	2077720	369044
Healthy member III-14	2458338	429397

InDels, short insertions and deletions.

Table 2
SNV and InDel associated with PC.

Gene	Chr	Gene Dir.	Chromosome position	RNA accession	SNP db_xref	Reference nucleotide	Genotype	Mutation call	Amino acid change
ADCY10	1	–	167849407	NM_018417.4		T	CT	c.[1162T>C]+[=]	388S>GS
NMU	4	–	56502274	NM_006681.2		A	insAGC	c.86_87insAGC	FS
SCN4A	17	–	62019300	NM_000334.4	rs121908544	G	GA	c.[4342G>A]+[=]	1448R>CR
PCSK4	19	–	1482365	NM_017573.3		C	delCT	c.1805_1806delCT	FS

Chr, chromosome; Gene Dir, gene direction; SNP, single nucleotide polymorphism; del, deletion; ins, insertion; FS, frameshift.

Because of the extremely low prevalence of PC in China, our results may help to make a contribution to build an epidemiological database for paramyotonia congenita. Our work is the first to systematically confirm that the genetic mutation in SCN4A is sufficient to lead to PC.

Materials and methods

Pedigree of the PC patients family

The affected family (Fig. 3), which originated from the Southwestern China pedigree (Chinese Han), had an onset in infancy. The proband (II-9) began with myotonia and weakness induced by cold temperatures in distal limb muscles and also showed ptosis. All of the patients exhibited similar clinical manifestations, but did not present any severe symptoms, such as amyotrophia and dyspnea.

Genomic DNA extraction from saliva of the PC patients

Genomic DNA was extracted from 2–5 ml saliva collected from the family members. After collection, 5 ml of Nuclei-SDS-ProteinaseK-Mastermix lysis buffer (NaCl 1.44 mM, Proteinase K 0.14 mg/ml, SDS 0.024 mM, EDTA 0.35 μM) was added immediately to the saliva sample. After incubation at 50 °C overnight, 3 ml saturated NaCl solution was added for salt precipitation, followed with ethanol precipitation. The DNA pellet was washed twice with 70% ethanol and dissolved in redistilled water.

Sequencing platform and bioinformatics software

For WES, the DNA coding sequence was enriched with the Agilent SureSelectXT Human All Exon kit. A sequencing library was prepared using the Illumina next generation sequencing kit. The Illumina HiSeq2000 sequencer (Illumina, San Diego, CA) was used for sequencing. The reads produced by sequencing were aligned for single nucleotide polymorphism (SNP) and InDel-calling using the NextGENe™ (SoftGenetics LLC) software. Raw data from the 7 samples (members with red asterisks in Fig. 3) were analyzed in NextGENe™ according to the manufacturer's standard analysis process.

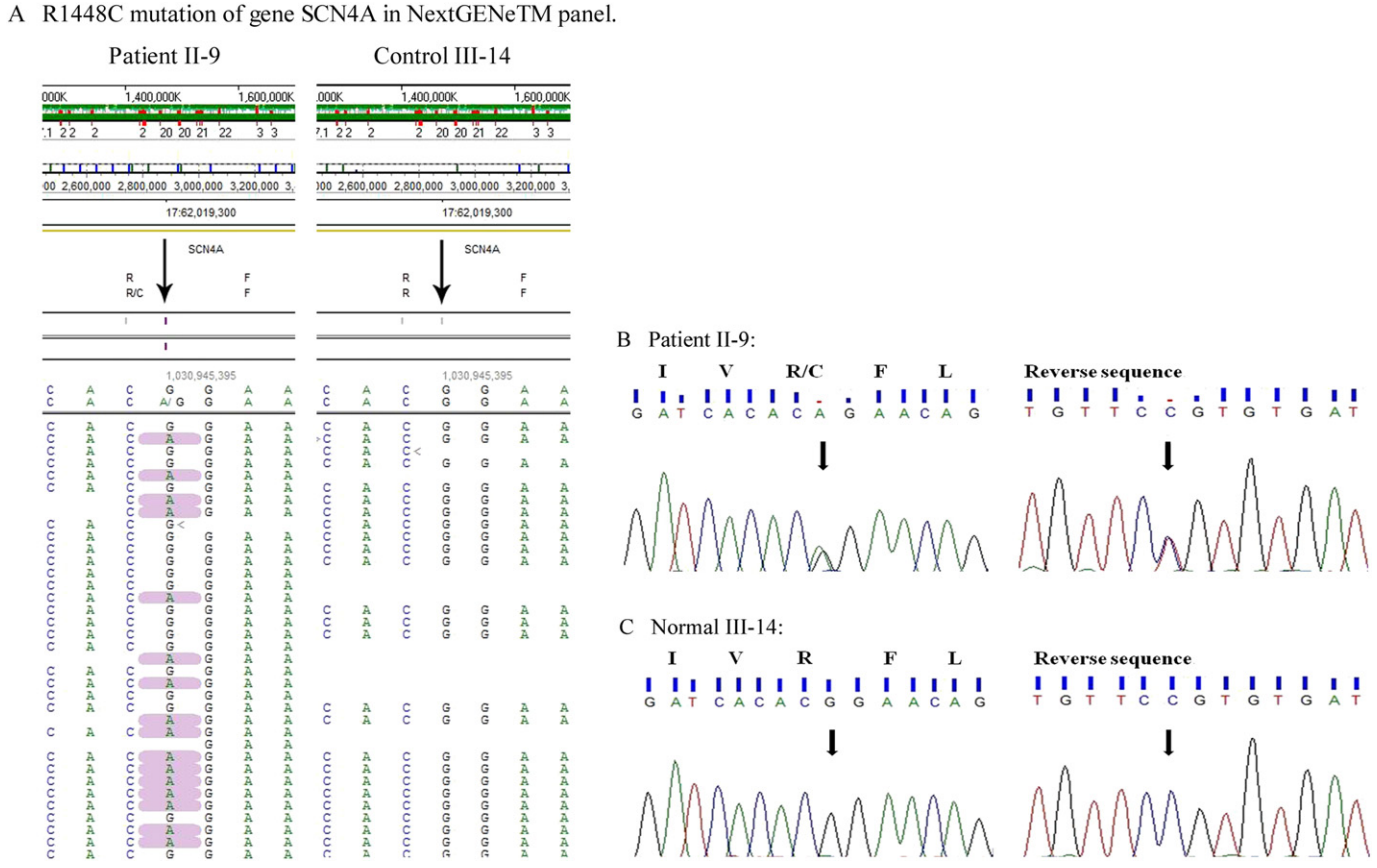

Fig. 2. R1448C mutation of gene SCN4A in NextGENe™ panel and confirmed by Sanger sequencing. (A) WES results were analyzed by the NextGENe™ software. After alignment and filtering, R1448C mutation of the gene SCN4A had been suspected to be the causative mutation. This figure showed the software panel of the R1448C mutation site. Left is Patient II-9, showing the CGT>TGT heterozygous mutation. Right is control III-14, and the sequence is consistent with the reference. (B) A missense mutation R1448C of the gene SCN4A was confirmed by Sanger sequencing in the proband (II-9) with heterozygous A/G. (C) Sanger sequencing of the R1448C-containing region from control (III-14).

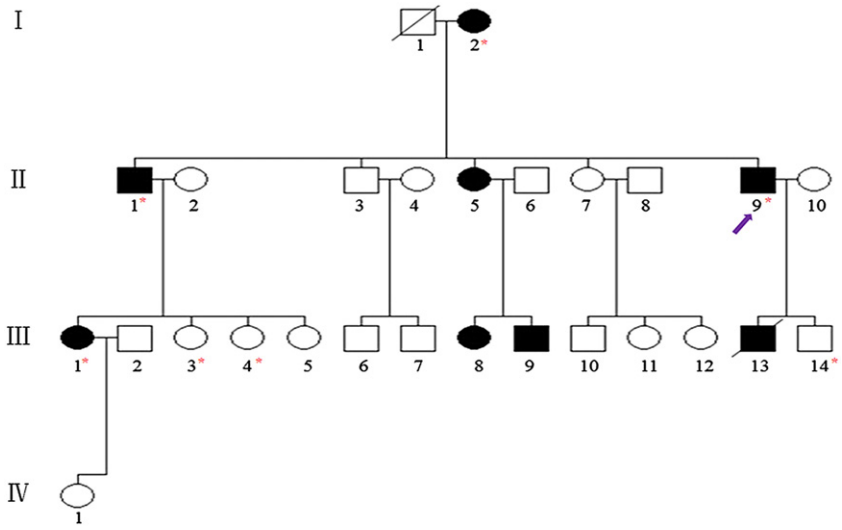

Fig. 3. Pedigree of the PC patients family. Males are represented by squares, females by circles, deceased by diagonals, and affected members by filled symbols. The proband (II-9) is indicated by an arrow. Asterisks (*) indicate that available DNA samples were used for WES. For simplicity, spouses and children of normal siblings in generations III are not shown. Also, only the affected individuals and individuals visited in this study are numbered in the pedigree tree. WES for II-3 has not been performed, but has completed subsequent genotyping for filtering.

Genetic information databases and bioinformatics tools for screening

A SNP detection and InDel-calling algorithm was run by high configuration servers. Nonsynonymous/splice acceptor and donor site/insertions or deletions in the dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), HapMap samples, and 1000 Genome (http://www.ncbi.nlm.nih.gov/Ftp/) with a frequency higher than 0.5% were removed. Synonymous changes and non-coding changes were filtered using the SIFT software (http://sift.jcvi.org). Sanger sequencing with specific primers was used to confirm the mutation from all of the samples collected from the family.

Acknowledgments

We would like to thank the patients and their families for their participation in this study. This work was supported by the National Natural Science Foundation of China (no. 81270295). Dr. Xiangdong Liu is a Fellow at the Collaborative Innovation Center for Cardiovascular Disease Translational Medicine and the recipient of Jiangsu Provincial Innovation fund.

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