Paramyotonia congenita: From clinical diagnosis to in silico protein modeling analysis

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学 位 論 文 の 内 容 要 旨

Paramyotonia congenita: From clinical diagnosis
to *in silico* protein modeling analysis

先天性パラミオトニア：臨床診断からコンピュータ予測によるタンパクモ
デル解析まで

神戸大学大学院医学研究科医科学専攻
疫学
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SUMMARY

Background:

Paramyotonia congenita (PMC) is an autosomal dominant disorder characterized by cold-or exercise induced myotonia. PMC is caused by a mutation in *SCN4A* which encodes the a-subunit of the skeletal muscle sodium channel. More than 50 different *SCN4A* mutations have been reportedm from several populations. Most of them are missense mutations. The diseases caused by the *SCN4A* mutations have diverse clinical phenotypes, not only PMC and SCM, but also other sodium channel. PMC and SCM can also be caused by mutations in the genes encoding channel proteins other than sodium channel. It has been reported that the human *CLCN1* gene in chromosome 7q35 that encodes the skeletal muscle chloride channel (CLC-1) was responsible for PMC

In this report, we present a Japanese girl with PMC who exhibited eyelid myotonia induced by cold exposure and repetitive blinking. Such symptoms were reproduced by orbital icepack and blinking tests. We analyzed her *SCN4A* and *CLCN1* genes and identified a disease-causing mutation in *SCN4A*. We also performed 3-D structure modeling to examine the mutation effect on the electrostatic surface charge and the conformational structure of the SCN4A protein.

Methods:

The patient was an 11-year-old Japanese girl who was diagnosed as having PMC on the basis of clinical findings, laboratory and electromyography examination. To confirm the diagnosis, an orbital ice-pack test and blinking tests were performed. Next, to identify the mutation, genetic analysis of *SCN4A* was performed. Parentage testing had also been performed to detect the presence of mutation in the family. Finally, to evaluate the mutation effect on the protein structure, *in silico* protein modeling analysis was performed.

Results and Discussion:

Cold-and exercise-induced myotonia was reproduced in the patient with non-invasive bed side tests: ice-pack and blinking tests. Molecular genetic analysis identified a missense mutation, c.4343G>A in *SCN4A* exon 24 in our patient, which led to the substitution of a highly-conserved arginine residue by a histidine at amino acid position 1448 of the SCN4A protein (p.Arg1448His). This finding confirmed the clinical diagnosis of PMC in our patient. Although PMC is known to be an autosomal dominant disorder, our patient was a sporadic case with no positive family history. The parentage test validated that the patient was the biological daughter of parents who carried no mutation in the *SCN4A* gene. These findings strongly suggested that the disease in the patient was caused by the mutation that might have occurred in the germ-line cells in one of the parents.

According to the protein modelling analysis, the p.Arg1448His mutation is located in the voltage-sensing transmembrane S4 segment of DIV (DIV/S4) of SCN4A. The mutation neutralized the positive electrostatic charge at 1448 in the DIV/S4 segment and disrupted the beginning of the helical structure in the DIV/S3-S4 linker of the SCN4A protein. According to an electrophysiological study of SCN4A by previous studies, the loss of highly conserved positive charge in DIV/S4 segment is associated with the slower fast inactivation of the mutant sodium channel p.Arg1448His than that of the wild-type channel. The slower fast inactivation of sodium channel may be the cause of PMC symptoms.

Conclusions:

Diagnostic physical interventions in the patient confirmed the phenotype presentation consistent with PMC, and the *in silico* protein modeling analysis of p.Arg1448His predicted structural changes which can affect function of the protein. All the data confirmed the diagnosis of PMC in the patient and added to existing literature emphasizing the important role of arginine residue at 1448.

論文審査の結果の要旨			
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論 文 題 目 Title of Dissertation	Paramyotonia congenita: From clinical diagnosis to *in-silico* protein modeling analysis 先天性パラミオトニア：臨床診断からコンピュータ予測による タンパクモデル解析まで		
審 査 委 員 Examiner	主　　査 Chief Examiner 副　　査 Vice-examiner 副　　査 Vice-examiner		

（要旨は１，０００字～２，０００字程度）

Background: Paramyotonia congenita (PMC) is an autosomal dominant disorder characterized by cold-or exercise induced myotonia. PMC is caused by a mutation in SCN4A which encodes the a-subunit of the skeletal muscle sodium channel. More than 50 different SCN4A mutations have been reportedm from several populations. Most of them are missense-mutations. The diseases caused by the SCN4A mutations have diverse clinical phenotypes, not only PMC and SCM, but also other sodium channel. PMC and SCM can also be caused by mutations in the genes encoding channel proteins other than sodium channel. It has been reported that the human CLCN1 gene in chromosome 7q35 that encodes the skeletal muscle chloride channel (CLC-1) was responsible for PMC. In this report, the authors including the candidate present a Japanese girl with PMC who exhibited eyelid myotonia induced by cold exposure and repetitive blinking. Such symptoms were reproduced by orbital icepack and blinking tests. The authors analyzed her SCN4A and CLCN1 genes and identified a disease-causing mutation in SCN4A. They also performed 3-D structure modeling to examine the mutation effect on the electrostatic surface charge and the conformational structure of the SCN4A protein.

Methods: The index patient was an 11-year-old Japanese girl who was diagnosed as having PMC on the basis of clinical findings, laboratory and electromyography examination. To confirm the diagnosis, an orbital ice-pack test and blinking tests were performed. Next, to identify the mutation, genetic analysis of SCN4A was performed. Parentage testing had also been performed to detect the presence of mutation in the family. Finally, to evaluate the mutation effect on the protein structure, in silico protein modeling analysis was performed.

Results and Discussion: Cold-and exercise-induced myotonia was reproduced in the patient with non-invasive bed side tests: ice-pack and blinking tests. Molecular genetic analysis identified a missense mutation, c.4343G>A in SCN4A exon 24 in our patient, which led to the substitution of a highly-conserved arginine residue by a histidine at amino acid position 1448 of the SCN4A protein (p.Arg1448His). This finding confirmed the clinical diagnosis of PMC in our patient. Although PMC is known to be an autosomal dominant disorder, our patient was a sporadic case with no positive family history. The parentage test validated that the patient was the biological daughter of the parents who carried no mutation in the SCN4A gene. These findings strongly suggested that the disease in the patient was caused by the mutation that might have occurred in the germ-line cells in one of the parents.

According to the protein modelling analysis, the p.Arg1448His mutation is located in the voltage-sensing transmembrane S4 segment of DIV (DIV/S4) of SCN4A. The mutation neutralized the positive electrostatic charge at 1448 in the DIV/S4 segment and disrupted the beginning of the helical structure in the DIV/S3-S4 linker of the SCN4A protein. According to an electrophysiological study of SCN4A by previous studies, the loss of highly conserved positive charge in DIV/S4 segment is associated with the slower fast inactivation of the mutant sodium channel p.Arg1448His than that of the wild-type channel. The slower fast inactivation of sodium channel may be the cause of PMC symptoms.

Conclusions: Diagnostic physical interventions in the patient confirmed the phenotype presentation consistent with PMC, and the in silico protein modeling analysis of p.Arg1448His predicted structural changes which can affect function of the protein. All the data confirmed the diagnosis of PMC in the patient and added to existing literature emphasizing the important role of arginine residue at 1448. ·

The candidate, having completed studies on molecular basis of paramyotonia congenita, with a specialty in mutation anlysis at the gene and protein levels, and having advanced the knowledge in genotype-phenotype correlations of the disease, is hereby recognized as having qualified for the degree of Ph.D.(Medicine).