An algorithm for candidate sequencing in non-dystrophic skeletal muscle channelopathies

Tai-Seung Nam · Christoph Lossin · Dong-Uk Kim · Myeong-Kyu Kim · Young-Ok Kim · Kang-Ho Choi · Seok-Yong Choi · Sang-Cheol Park · In-Seop Na

Received: 13 September 2012 / Revised: 9 January 2013 / Accepted: 9 February 2013 / Published online: 3 March 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract Human skeletal muscle channelopathies (HSMCs) are a group of heritable conditions with ion channel–related etiology and similar presentation. To create a comprehensive picture of the phenotypic spectrum for each condition and to devise a strategy that facilitates the differential diagnosis, we collected the genotype and phenotype information from more than 500 previously published HSMC studies. Using these records, we were able to identify clear correlations between particular clinical features and the underlying alteration(s) in the genes SCN4A, CACNA1S, KCNJ2, and CLCN1. This allowed us to develop a clinical, symptom-based, binary decision flow algorithm that predicts the proper genetic origin with high accuracy (0.88–0.93). The algorithm was implemented in a stand-alone online tool ("CGPS"—http://cgps.ddd.co.kr) to assist with HSCM diagnosis in the clinical practice. The CGPS provides simple, symptom-oriented navigation that guides the user to the most likely molecular basis of the presentation, which permits highly targeted genetic screens and, upon confirmation, tailored pharmacotherapy based on the molecular origin.

Keywords Channelopathy · Skeletal muscle · Algorithm · Periodic paralysis · Myotonia

Introduction

Human skeletal muscle channelopathies (HSMCs) are a heterogeneous group of seven heritable non-dystrophic disorders, including autosomal dominant and recessive congenital myotonia (OMIM255700 and 160800, respectively), paramyotonia congenita (168300), sodium channel myotonia (also: potassium-aggravated myotonia or PAM—608390), hyper- and hypokalemic periodic paralysis (170500, 170400, 613345), and Andersen–Tawil syndrome (170390); all of them are caused by ion channel dysfunc-
tion. While the phenotype of each disorder is unique, almost all HSMCs present with either myotonia (i.e., an abnormal delay in muscle relaxation) or periodic paralysis [6], which can complicate the differential diagnosis. Sequencing of candidate genes has therefore become common clinical practice to reconfirm the phenotypic diagnosis. HSMCs with myotonia are frequently linked to genetic alterations in CLCN1 (protein name: ClC-1) or SCN4A (protein: Na^+4,1.4), while hyperkalemic periodic paralysis (HyperPP), hypokalemic periodic paralysis (HypoPP), and Andersen–Tawil syndrome commonly stem from mutations in SCN4A, CACNA1S (protein: Ca^2+1.1), and KCNJ2 (protein: K^+2.1), respectively [8, 10]. Unfortunately, there is no firm phenotype/genotype relationship. HypoPP, for example, can be caused by mutations in CACNA1S, KCNJ2, or SCN4A, but changes in SCN4A can also give rise to paramyotonia congenita (PMC), sodium channel myotonia, or HyperPP [6, 8, 10]. This heterogeneity does not permit a reliable prediction as to the molecular origin of the presentation. Non-targeted full sequencing of all four candidate genes is out of the question, as they range in size from approximately 15.5–78.1 kb. Sequencing only the exonic regions of the four genes does little to improve the situation, because the combined coding length is in excess of 15.3 kb. Next-generation sequencing will eventually allow for comprehensive genetic testing. At the current time, however, whole-genome sequencing and whole-exome sequencing remain technologies that are too costly for routine analyses regarding missense mutations. It is therefore useful to identify pointer symptoms that provide directions regarding the molecular origin of the pathology. We hypothesized that, rather than attempting to correlate any one HMSC condition as a whole with a particular genetic defect, considering the individual set of copresenting symptoms may provide stronger prediction power in terms of the underlying genetic abnormality. This would lower the risk of misdiagnosis, enhance diagnostic efficiency, and help elevate therapy from a condition-based approach to patient-tailored treatment.

Subjects and methods

Data collection strategy

Our meta-analysis targeted all English-language literature on genetically confirmed HSMCs (i.e., OMIM records #160800, 168300, 170500, 170400, 170390, 608390, 613345) up to November 2010. The search strings “myotonia”, “periodic paralysis”, “SCN4A”, “CACNA1S”, “CLCN1”, or “KCNJ2” were run against various databases, including the US National Library of Medicine (PubMed—http://www.ncbi.nlm.nih.gov/pubmed/), the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/), Wikipedia (http://en.wikipedia.org/wiki/), and Online Mendelian Inheritance in Man (OMIM—http://www.ncbi.nlm.nih.gov/omim/). To minimize the numeric bias of repeatedly reported variations with the same phenotype, individuals and families with the same clinical and genetic features reported by the same author(s) were treated as one case. Conversely, any family members deviating from the common phenotype were treated as separate cases. Studies involving thyrotoxic HypoPP, and KCNE3-related pathologies were excluded. The study protocol was approved by the Ethical Committee of Chonnam National University Medical School, and was carried out in accordance with the 1975 Declaration of Helsinki.

Study variables

All data were entered into a standardized form (eFigure 1) to generate comparable variables. Taken into consideration were epidemiologic data, inheritance pattern, original clinical impression, chief complaints and their relevant laboratory data [e.g., age of onset, severity, duration, affected body part, triggers and relief, treatment response, presence/absence of the myotonic warm-up phenomenon, paramyotonia (paradoxical myotonia), percussion myotonia, electromyography and electrocardiography data, muscle biopsy, etc.], as well as the genotype. Topological information (e.g., channel domain, transmembrane region, etc.) as well as additional data (e.g., biochemical severity of the amino acid change, electrophysiological analyses, etc.) were recorded where possible. Values for routinely reported clinical variables were rated as “negative” (i.e., symptom not present) when the actual value was not reported, based on the premise that an abnormality would have carried sufficient diagnostic importance to warrant mentioning in the publication (e.g., a study’s EKG was rated as “normal” unless ventricular arrhythmia or QT prolongation [2, 11] was specifically mentioned). Likewise, developmental dysmorphism was rated “absent” unless clindactyly, hypertelorism, micrognathia, low-set ears, broad forehead, or a cleft lip/palate were noted; stiffness and myalgia were treated in the same fashion. In some cases, values for variables not routinely reported had to be excluded (e.g., muscle pathology, such as pericritic potassium or creatine kinase levels and electromyographic findings).

Statistical analysis

Data analysis utilized SPSS Statistics (IBM, Chicago, USA). Clinical features were treated as independent variables, while genotype data were treated as dependent. We employed the Pearson’s Chi-square test to identify distinct
clinical features that roughly discriminated between channelopathies. Probability values of $p < 0.01$ were deemed statistically significant.

Supervised machine learning and algorithm development

Randomly selected cases equaling 60% of the data pool were used to develop the decision diagrams (Figs. 1, 2); the remaining 40% helped test the performance of the implied algorithms, according to the principle of supervised machine learning [1, 3]. For SCN4A channelopathies, the clinical features were additionally correlated with the topological location of the affected amino acid residue within the channel protein. Some of the records initially collected (e.g., age of onset, duration, severity, drug response, and frequency of attacks) had to be excluded due to lack of standardization between the different studies.

Individual algorithms were generated for the two chief clinical complaints in the HSMCs, which were periodic paralysis and myotonia. An effort was made to identify the smallest possible number of relevant features leading to satisfactory algorithm performance. The associated ranking order in the decision diagram takes four parameters into account, which are (1) linkage, (2) statistical significance, (3) redundancy, and (4) coupling. Linkage, operationally defined in the context of this manuscript as the exclusive presentation of a clinical feature with a specific genotype, was given the highest priority. This produced, e.g., the (paramyotonia/SCN4A) and (recessive inheritance/CLCN1)
connections for the myotonia flow chart (Fig. 2). Statistical significance, as assessed by variables with smaller p-values, was deemed to have superior prediction power and therefore ranked higher in priority. Redundancy, in other words uninformative or inconsistently reported variables, was eliminated where possible. Clinical features that emerged as always co-presenting were deemed “coupled” and subsequently handled as one [e.g., (dysmorphism/abnormal EKG) predicting KCNJ2 association in Fig. 1].

Results

Data collection

We reviewed over 500 HSMC studies; 205 studies involved SCN4A, 69 reported on CACNA1S, 91 related to CLCN1, and 141 publications had ties to KCNJ2. The extracted data were genotype-phenotype correlated, which combined 628 genetically confirmed HSMC cases from 585 families with a total of 1,082 affected members.

Phenotype-genotyping algorithm

All patients presented with episodic paralysis (41.3 %), myotonia (64.5 %), or both (23.2 %) on their first exam. Periodic paralysis was commonly associated with genetic alterations in SCN4A (43.2 %), CACNA1S (39.8 %), or KCNJ2 (16.2 %). Myotonia, on the other hand, was essentially exclusive to changes in CLCN1 and SCN4A (69.1 and 30.6 %, respectively, Table 1). This prompted us to develop two separate algorithms for, what we deemed, the two chief clinical features, periodic paralysis and myotonia, each with its own set of secondary clinical features capable of discriminating between the different causative genes.

Diagnostic algorithm in presence of EPISODIC PARALYSIS (Fig. 1; Table 2)

In patients with periodic paralysis, the most predictive secondary clinical feature was hyperkalemia, which suggested genetic defects in SCN4A. Dysmorphism and EKG abnormality, on the other hand, both segregated with KCNJ2 abnormality and were hence treated as one variable. Stiffness and myalgia were coupled in a similar fashion, albeit with genetic alterations in SCN4A. Patients who copresented periodic paralysis and myotonia had a high likelihood for SCN4A alterations. Several other clinical features were promising in terms of predicting the causative gene, but ultimately had to be excluded because too many original reports (>50 %) had not reported them.

Diagnostic algorithm in presence of MYOTONIA (Fig. 2; Table 3)

In patients with myotonia, recessive inheritance and paramyotonia surfaced as unique clinical features for CLCN1 and SCN4A channelopathy, respectively. Other clinical features with indicator status in this second algorithm were warm-up (linking to CLCN1), periodic paralysis (SCN4A), and eyelid/bulbar myotonia (SCN4A). Cold-sensitive myotonia and percussion myotonia may harbor additional prediction power, but had to be excluded because of a

Table 1 Genetic incidence for the two chief clinical features, PERIODIC PARALYSIS and MYOTONIA

<table>
<thead>
<tr>
<th>Chief clinical feature</th>
<th>Total</th>
<th>Implicated gene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SCN4A</td>
</tr>
<tr>
<td>Periodic paralysis (n = 258)</td>
<td>41.1%</td>
<td>39.9%</td>
</tr>
<tr>
<td></td>
<td>(258/627)</td>
<td>(103/258)</td>
</tr>
<tr>
<td>Myotonia (n = 404)</td>
<td>64.4%</td>
<td>0.2%</td>
</tr>
<tr>
<td></td>
<td>(404/627)</td>
<td>(1/404)</td>
</tr>
<tr>
<td>Simultaneous presentation (n = 146)</td>
<td>23.3%</td>
<td>0.7%</td>
</tr>
<tr>
<td></td>
<td>(146/627)</td>
<td>(1/146)</td>
</tr>
<tr>
<td>Patient percentage, total, with indicated gene defect</td>
<td>16.6%</td>
<td>6.7%</td>
</tr>
<tr>
<td></td>
<td>(103/627)</td>
<td>(42/627)</td>
</tr>
</tbody>
</table>

Tabulation of the clinical presentation for 627 patients revealed that two symptoms, periodic paralysis and myotonia, were present at all times. Cross-correlation with the underlying genetic defect produced probability values of $p < 0.001$, implying that both symptoms have excellent ability to differentiate between the different causative genes. Note that the reported percentages do not represent the chance of a given gene’s likelihood to be involved, but the percentage of patients with the indicated symptom compared to the total number of patients with a particular symptom (e.g., 103 patients had a genetic defect in CACNA1S; this constitutes 39.9 % vs. the total number of patients with periodic paralysis, which was 258). The sum of a column’s percentages does not add up to 100 % because some patients presented with both, periodic paralysis and myotonia. Reference sequences for the genes are: CACNA1A—NG_009816.1/NM_000069.2, KCNJ2—NG_008798.1/NM_000891.2, SCN4A—NG_011699.1/NM_000334.4, and CLCN1—NG_009815.1/NM_000832. Dashes indicate zero cases.
strong correlation with the already selected chief clinical features or due to a large number of missing values from the original reports.

Phenotype/topology correlation for SCN4A channelopathy (Fig. 3; Table 4)

Clear phenotype-genotype correlations in the SCN4A channelopathies allowed us to develop an algorithm that extended the genetic testing prediction power into the subdomains of the Na\textsubscript{v}1.4 protein. The second and fourth homologous domains (D2 and D4, respectively) of the Nav1.4 protein stood out as mutation hot spots: Of the 49 SCN4A mutations included in the study, 27 (55.1\%) were located in D2 or D4, which occurred in 104/172 families. Arginine 1,448 of D4 was the strongest contender for substitution; it was altered in 23 families. Next likely to be exchanged were residues 704 in D2 and residue 1,313 in the D3–D4 cytoplasmic linker (both in 19 families). Aside from 1,313, the D3–D4 linker harbored two further sites that were mutated in 16 HSMC families. Comparatively rare were changes D1 and D3, with 9 sites in 15 families, and 8 sites in 16 families, respectively. The cytoplasmic D1–D2 linker and the C-terminus were altered each only once [5, 14]. The associated clinical features and their frequencies are listed in Table 4; symptoms with predictor status are compiled in Fig. 3.

The remaining genes, CACNA1S, CLCN1 and KCNJ2, were analyzed in a similar fashion, but the results were not as informative as for SCN4A. In the calcium channel gene,
most mutations were in D4 or D2 (95.6 %), with hotspots at positions 1,239 and 528, which were mutated in 52.5 and 43.1 % of families carrying CACNA1S mutations. Three further variations located to positions 876, 897, and 900 in D3, and two were intronic. Genetic alterations in the CLCN1 gene showed no topological preference other than a concentration around exon 8, which was found in 37.4 % of families with a CLCN1 mutation. In patients where KCNJ2 was altered, the mutations mapped to the termini (both C and N) as well as to the P-loop.

Table 4  Phenotype/topology correlation for SCN4A channelopathy

<table>
<thead>
<tr>
<th></th>
<th>D1 (n = 13)</th>
<th>D2 (n = 68)</th>
<th>D3 (n = 16)</th>
<th>D4 (n = 56)</th>
<th>D3–D4 linker (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Independent 1 paralysis</td>
<td>30.8 %</td>
<td>91.2 %</td>
<td>75 %</td>
<td>48.2 %</td>
<td>18.2 %</td>
</tr>
<tr>
<td>Independent 1 myotonia</td>
<td>84.6 %</td>
<td>30.9 %</td>
<td>37.5 %</td>
<td>91.1 %</td>
<td>100 %</td>
</tr>
<tr>
<td></td>
<td>(11/13)</td>
<td>(21/68)</td>
<td>(6/16)</td>
<td>(51/56)</td>
<td>(33/33)</td>
</tr>
<tr>
<td>Simultaneous periodic paralysis and myotonia</td>
<td>23.1 %</td>
<td>26.5 %</td>
<td>12.5 %</td>
<td>62.5 %</td>
<td>30.3 %</td>
</tr>
<tr>
<td></td>
<td>(3/13)</td>
<td>(18/68)</td>
<td>(2/16)</td>
<td>(35/56)</td>
<td>(10/33)</td>
</tr>
<tr>
<td>Pericritic hypokalemia</td>
<td>50 %</td>
<td>41.2 %</td>
<td>50 %</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>(2/4)</td>
<td>(21/51)</td>
<td>(5/10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pericritic normokalemia</td>
<td>50 %</td>
<td>37.3 %</td>
<td>20 %</td>
<td>62.5 %</td>
<td>100 %</td>
</tr>
<tr>
<td></td>
<td>(2/4)</td>
<td>(19/51)</td>
<td>(2/10)</td>
<td>(10/16)</td>
<td>(6/6)</td>
</tr>
<tr>
<td>Pericritic hyperkalemia</td>
<td>–</td>
<td>21.6 %</td>
<td>30 %</td>
<td>37.5 %</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(11/51)</td>
<td>(3/10)</td>
<td>(6/16)</td>
<td></td>
</tr>
<tr>
<td>Paramyotonia</td>
<td>33.3 %</td>
<td>31.6 %</td>
<td>–</td>
<td>69.6 %</td>
<td>88.2 %</td>
</tr>
<tr>
<td></td>
<td>(1/3)</td>
<td>(6/19)</td>
<td></td>
<td>(16/23)</td>
<td>(15/17)</td>
</tr>
</tbody>
</table>

A compilation of clinical features observed in patients with confirmed genetic alterations in the SCN4A gene. D1–D4 refer to the respective homologous protein domains. Reference sequence was NP_000325.4 with the following boundaries: D1 = 129–449, D2 = 574–802, D3 = 1,027–1,295, and D4 = 1,349–1,598. Dashes indicate zero cases

1 The qualifier “independent” indicates separate occurrence of myotonia and periodic paralysis as opposed to these two clinical features presenting at the same time.
Algorithm performance

With the algorithms established using 60% of our patient pool, we conducted a performance evaluation using the remaining 40% of the cases. This showed that, for the two chief complaints of periodic paralysis and myotonia, the correct genetic location could be predicted with accuracies of 0.93 and 0.88, respectively. In terms of predicting the exact genetic location in periodic paralysis, accuracies of 0.95, 1.0, and 0.84 were obtained for genes CACNA1S, KCNJ2, and SCN4A, respectively (Fig. 1). The performance was similarly satisfying in the myotonia algorithm (Fig. 2), which differentiated between CLCN1 and SCN4A with accuracies of 1.0 and 0.76, respectively.

Computer-assisted genetic screening

Our analyses revealed several clinical features with strong statistical power of differentiating between different HSMCs. This prompted us to devise a computer-assisted user interface that generates recommendations for genetic screening based on user responses to simple questions regarding the patient’s clinical presentation. An online version of this tool is available at http://cgps.ddd.co.kr.

Discussion

The present study re-examined the HSMC phenotype–genotype correlation with a systematic review of the medical literature on genetically confirmed HSMCs. The primary finding in this meta-analysis of over 500 publications was a simple, binary decision flow that can substantially simplify diagnosis and suggest, with high accuracy, what gene to sequence for molecular confirmation. Indeed, our analyses revealed sufficient information to allow for the targeted analysis of specific gene subregions when SCN4A was involved; an obvious advantage in terms of genetic screening efficiency.

A secondary finding was that the current clinical HSMC classification and treatment guidelines are in need of revision. This is based on three considerations. First, the phenotype-genotype correlations are evidently more complex than what the current standards assume. One example is the connection between HypoPP and CACNA1S. While we confirmed that HypoPP is indeed most commonly precipitated by alterations in CACNA1S, we found that SCN4A and KCNJ2 must be examined as well, especially when only few distinctive clinical features are present (Table 2) aside from altered serum potassium concentration with muscle weakness. Warm-up and temperature-sensitive myotonia produced similar genetic heterogeneity (Table 2; Fig. 2).

Second, HSMC classification based only on phenotype is insufficient; the results from genetic screenings must be taken into account. The warm-up phenomenon, e.g., has traditionally been treated as a sure indicator of CLCN1 mutation in congenital myotonia [6, 8, 9]. However, Trip and colleagues [13] found warm-up in three patients with an SCN4A mutation. In fact, in some cases, the clinical features can be so mixed—one report exists with a presentation reminiscent of PMC, HyperPP, and MC—that a differential can only be reached with genetic sequencing results [7].

Third, differentiating HSMCs clinically may carry no significant benefit. Paramyotonia in PMC, e.g., is a very interesting phenomenon clinically, especially when compared to warm-up myotonia in PAM. However, there is no distinctive management strategy for these symptoms. Additionally, potassium sensitivity is not a feature specific to sodium channel myotonia, but an occurrence common to all SCN4A channelopathy [9]. What is more, some formerly phenotypically distinct disorders, such as HyperPP and PMC, now have such an overlapping clinical presentation that a differentiation may no longer be possible [4]. Genetically, however, both paramyotonia and pericritic hyperkalemia, strongly point toward SCN4A channelopathy rather than other genes (Table 2; Fig. 2).

Taking these considerations into account, we see it warranted that the clinical classification for HSMC be reorganized using a new system that includes information on genetic etiology. The advantage of a genetically-amended classification system is its integration of ion channel pathophysiology, which will encourage functional analyses at the molecular level that can drive the development of new therapies. The notion of adding genetic data to the phenotypic diagnosis is not new. Raja Rayan and Hanna [10] recently introduced an algorithm for genetic testing in myotonia and periodic paralyses based on a meticulous review of the recent findings in genetics, pathophysiology, phenotypy, and treatment. Similar to the approach used in this study, the procedure relies on distinctive clinical features that suggest a causative gene. This is useful for cases where the clinical presentation is unambiguous. For less common phenotypes (e.g., satisfying only two out of three criteria), however, coming to the appropriate conclusion may become challenging. Our algorithms are more flexible, in that do not require rigid sets of symptoms, as they lead the user with a binary yes/no decision flow to the final answer. In another study, Trip and co-workers [12] developed a guideline for genetic testing for myotonia, based on standard bedside tests. This can be very practical to differentiate SCN4A from CLCN1 myotonia. Our study extends this approach and adds topological detail for more targeted genetic testing, as well as differentiation of several other, phenotypically similar conditions with defects in CACNA1S and KCNJ2.
There are limitations to our analyses. First, we limited ourselves to English literature reports. It is conceivable that certain, geographically/ethnically-isolated areas where English does not serve as the official language present with a different genetic constellation when it comes to HSAMCs. Inclusion of non-English publications may therefore sway our algorithms in a different direction, or conversely, support them. Second, we used the same data collection procedure for all publications, but not all data could be standardized. This led to the exclusion of commonly, but not always, reported clinical features such as muscular hypertrophy and age of onset. It is also true that HSMC studies without genetic information—excluded by virtue of incompatibility with our analysis approach—represent a limitation to our analyses. The findings from these cases, if re-evaluated genetically in the future, may provide additional information requiring adjustments in our model.

Finally, we should caution that other, unrelated muscle disorders exist (e.g., myasthenia gravis, episodic ataxia, etc.) that, on rare occasions, may present with clinical features similar to the conditions discussed here.

In summary, we introduce clinically oriented algorithms that provide guidance for genetic testing in HSMC; an online platform has been set up (http://cgps.ddd.co.kr) to provide universal access for clinicians. Our analyses prompt us to recommend a review of the current classification standards and therapy recommendations for HSAMCs. We further suggest adoption of a standardized phenotype/genotype reporting method to simplify the integration of data from new HSMC reports into their respective locus-specific databases.

Acknowledgments This work was supported by a grant of the Korean Health Technology R and D Project, Ministry for Health, Welfare and Family Affairs, Republic of Korea (A100402).

Conflicts of interest The authors declare that they have no conflict of interest.

Glossary

CACNA1S  Gene, calcium channel Ca_{v}1.1
CLCN1  Gene, human skeletal muscle chloride channel CIC-1
HSMC  Human skeletal muscle channelopathy
Hyper-/Hypo-/NormoPP  Hyper-/Hypo-/Normokalemic periodic paralysis
KCNJ2  Gene, inward-rectifier potassium ion channel K_{ir}2.1
OMIM  Online Mendelian Inheritance in Man (http://us-east.omim.org/)

References


PAM  Potassium-aggravated myotonia
PMC  Paramyotonia congenita
SCN4A  Gene, human skeletal muscle sodium channel Na_{v}1.4