Prevalence of genetically confirmed Skeletal Muscle Channelopathies in the era of next generation sequencing.

Vinojini Vivekanandam[1], Rawan Jaibaji[2], Richa Sud[1], Rebecca Ellmers[1], Iwona Skorupinska[1], Louise Germaine[1], Natalie James[1], Sarah Holmes[1], Roope Manniko[1], Dipa Jaysaeelan[1], Michael G Hanna[1]

1. Centre for Neuromuscular Disorders, UCL QS Institute of Neurology, Queens Square, London, WC1N 3BG
2. University College London, London

Corresponding author:

Dr. Vinojini Vivekanandam

v.vivekanandam@ucl.ac.uk

Box102

Centre for Neuromuscular Diseases

National Hospital for Neurology and Neurosurgery

London, UK

WC1N 3BG

Highlights

- The current minimum point prevalence for all skeletal muscle channelopathies is 1.99/100 000.
- The minimum point prevalence of myotonia congenita is 1.13/100 000; for paramyotonia congenita/sodium channel myotonia is 0.35/100 000, for hypoPP/hyperPP is 0.41/100 000 and for Andersen Tawil Syndrome is 0.1/100 000.
- Next generation sequencing as well as increased referral rates have been primary contributors to the almost doubled point prevalence rate from 1.12/100 000 to 1.99/100 000.

Abstract:

We provide an up-to-date and accurate minimum point prevalence of genetically defined skeletal muscle channelopathies which is important for understanding the population impact, planning for treatment needs and future clinical trials. Skeletal muscle channelopathies include myotonia congenita (MC), sodium channel myotonia (SCM), paramyotonia congenita (PMC), hyperkalemic periodic paralysis (hyperPP), hypokalemic periodic paralysis (hypoPP) and Andersen- Tawil Syndrome (ATS). Patients referred to the UK national referral centre for skeletal muscle channelopathies and living in UK were included to calculate the minimum point prevalence using the latest data from the Office for National Statistics population estimate. We calculated a minimum point prevalence of all skeletal muscle channelopathies of 1.99/100 000 (95% CI 1.981-1.999). The minimum point prevalence of MC due to *CLCN1* variants is 1.13/100 000 (95% CI 1.123-1.137), *SCN4A* variants which encode for PMC and SCM is 0.35/100 000 (95% CI 0.346 – 0.354) and for periodic paralysis (HyperPP and HypoPP) 0.41/100 000 (95% CI 0.406-0.414). The minimum point prevalence for ATS is 0.1/100 000 (95% CI 0.098-0.102). There has been an overall increase in point prevalence in skeletal muscle channelopathies compared to previous reports, with the biggest increase found

to be in MC. This can be attributed to next generation sequencing and advances in clinical, electrophysiological and genetic characterisation of skeletal muscle channelopathies.

Keywords: Channelopathy, Myotonia Congenita, Periodic Paralysis, Andersen Tawil Syndrome, Muscle channelopathy

Introduction:

Skeletal muscle channelopathies are a group of inherited neuromuscular disorders caused by variants in genes that encode for ion channels. They consist of a group of rare episodic genetic disorders comprising of non-dystrophic myotonias (NDM) and periodic paralyses (PP) and can range from mild symptoms to causing significant morbidity. The non-dystrophic myotonias are myotonia congenita (MC), paramyotonia congenita (PMC) and sodium channel myotonia (SCM). The periodic paralyses are hypokalemic periodic paralysis (hypoPP), hyperkalemic periodic paralysis (hyperPP) and Andersen-Tawil Syndrome (ATS). These particular phenotypes are inherited in either an autosomal dominant or de novo fashion, with the exception of MC, which can be inherited as either dominant or recessive[1].

Significant progress has been made in the characterisation of skeletal muscle channelopathies since the first UK prevalence study (2011) which calculated the minimum point prevalence at 1.12/100,000[2]. We have since characterised 95 chloride channel (ClC-1) variants in 223 probands electrophysiologically[3]. We have also phenotyped the largest international cohort of patients with ATS, publishing eight novel variants we characterised electrophysiologically[4]. Since 2011, there has been widespread adoption of next generation sequencing in the national referral centre for skeletal muscle channelopathies at the National

Hospital for Neurology and Neurosurgery, and an increase in knowledge of pathogenic variants. We obtain an updated prevalence rate of these conditions in this new era.

Methods:

We analysed patients on the National Channelopathy database who were referred for clinical or genetic assessment between 1997 and 2021. Since the configuration of the NHS Genomic Medicine Service in January 2019, all testing in UK for the skeletal muscle channelopathy panel is by performed by our service. Prior to this, the majority of testing for muscle channelopathy panel was provided by our service as we are the National Commissioned Highly Specialised Service for Muscle Channelopathies.

Patients included were of any age, living in the UK and had a clinical phenotype in keeping with NDM or PP. Patients with other causes such as myotonic dystrophy or thyrotoxic PP were excluded.

Patients then underwent genetic confirmation and were included in the study if they carried pathogenic variants in *CLCN1, SCN4A, CACNA1S* or *KCNJ2*. Genetic testing methodology is detailed in prior reports[3]. In brief, patients who had exome sequence as well as next generation sequencing were included. Prior to 2013, sequencing of the coding region of *CLCN1* or *KCNJ2* was performed while sequence analysis of commonly mutated *SCN4A* or *CACNA1S* exons was undertaken and, if negative, the remaining coding regions were sequenced. More recently, next generation sequencing was undertaken with parallel sequencing of DNA strands via a gene panel consisting of initially a four gene panel (*CLCN1, SCN4A, CACNA1S, KCNJ2*) and then expanded to a seven gene panel (*CLCN1, SCN4A, CACNA1S, KCNJ2*, *ATP1A2*, *SLC2A1, CACNA1A*).

Methods for generation of channel variants, expression of channel variants in Xenopus oocytes, electrophysiological analysis using two-electrode voltage-clamp and criteria for determination of pathogenicity were recently described and were combined with clinical data[3].

The latest UK population was taken from the Office for National Statistics (ONS) UK population estimate published in June 2021, which was noted to be 67,081,000[5]. The minimum point prevalence was calculated through dividing the number of patients with genetically defined skeletal muscle channelopathy by the total at risk population. The prevalence was then subsequently expressed per 100,000 population. The frequency of individual variants was also calculated. The confidence intervals were subsequently calculated using Poisson distribution. Excel version 16.65 and IBM SPSS version 26 were used to present descriptive data.

Results:

We reviewed a total of 4241 tested patients on the channelopathy database. There were 871 patients with *CLCN1* variants, 244 with *SCN4A* variants, 67 with *KCNJ2* variants 127 with *CACNA1S* variants. The current minimum point prevalence for all skeletal muscle channelopathies is 1.99/100 000 (95% CI 1.981-1.999).

The minimum point prevalence of MC is 1.13/100 000 (95% CI 1.123-1.137), table 1. The mean age of patients with MC currently in the population is 37.6 years. A total of 128 *CLCN1* variants were detected. 17 variants accounted for approximately 70% of all cases of myotonia congenita, the most common *CLCN1* variants are shown in table 2. The most common variant is *CLCN1* Gly230Glu.

In PMC and SCM encoded for by variants in *SCN4A*, the minimum point prevalence is 0.35/100 000 (95% CI 0.346 – 0.354) detected in 58 confirmed pedigrees. Eight variants accounted for 84% of cases, the most common being *SCN4A* Thr1313Met.

For hypoPP and hyperPP combined phenotypes, the minimum point prevalence is 0.41/100,000 (95% CI 0.406-0.414) in 58 confirmed pedigrees. Four variants accounted for approximately 75% of the cases, table 3. The minimum point prevalence of ATS is 0.1/100 000 (95% CI 0.098-0.102) with 27 variants in 49 confirmed pedigrees. The seven most common variants accounted for half the cases of ATS, table 3.

We additionally identified patients with "double trouble" variants. Five patients with *CLCN1* variants additionally had *SCN4A* variants. Two of these *SCN4A* variants (Gly452Lys and Glu460Lys) are variants of uncertain significance, the other three *SCN4A* Ala1156Thr, Val1293Ile and Asp1309Glu are known to be pathogenic. Two patients with a pathogenic *CLCN1* variant additionally had a myotonic dystrophy type 2 expansion.

Discussion:

We provide an up-to-date minimum point prevalence estimate of skeletal muscle channelopathies and their subsets in the UK. The prevalence has almost doubled since the last report in 2011 likely to be largely due to the advent of next generation sequencing. While sanger sequencing is accurate, it is costly and time consuming. Next generation sequencing allows massively parallel DNA sequencing which has improved diagnostic rates, times and costs.

Moreover, we have developed an extensive electrophysiological database allowing characterisation of genetic variants. Electrophysiological techniques have improved as have

our physiological understanding of disease mechanisms. Our multi-disciplinary approach allows accurate classification using the American College of Medical Genetics (ACMG) genetic criteria[6]. We have also seen an increase in referral rates to the national referral centre. This may be explained by increased awareness and recognition of skeletal muscle channelopathies as well as an increase in treatment options for patients over time.

Diagnosis is important as there are treatment options that are available for symptomatic management for patients. Additionally due to the cardiac risk seen in Andersen-Tawil syndrome, cardiac screening and family testing is paramount[4].

In addition to advances in genetic technique, clinician awareness of muscle channelopathies has improved. In particular, understanding of paediatric syndromes and nuances of the clinical spectrums have improved referral rates and diagnostic rates[4,7,8].

This is a minimum prevalence as only patients seeking medical attention have been genetically analysed. It is likely that mild affected patients or family members have not sought testing making the true prevalence of muscle channelopathies greater. Moreover, a small subset of patients prior to 2019 may not have been tested by our lab and hence not be included in our analysis. While we are the national referral centre, few remote centres prior to 2019 did offer testing and these patients would be missed in our database.

An additional limitation of the study includes the use of cumulative data from 1997 to 2021 with a minimum point prevalence calculated using population statistics from 2021 which may not apply to study data prior to 2021.

We found a slightly higher number of autosomal dominant MC in our cohort (343 vs 225) in the common variants which is in keeping with the characteristics of our cohort we reported in

2011. We have identified several new variants since 2011 which is likely due to yield of next generation sequencing.

Interestingly, a small number of patients had two variants identified, and in the patients where both variants were pathogenic, the phenotype was altered. We have previously described these cases and it is clinically important to consider this in atypical or more severe phenotypes of skeletal muscle channelopathies[7,9].

Our reported point prevalence of 1.99/100 000 is comparable to other reports in smaller studies – 2.38/100 000 reported in the Netherlands (2015-2018)[10]. The most frequent *CLCN1* variant seen in the Dutch population was Gly285Glu (seen in 26 patients). While Gly230Glu (seen in 154 patients in our cohort) was not reported at all in the Dutch cohort. There may be higher frequencies of carrier mutations in the Dutch cohort and it is likely that such differences in genetic variants exist across the world. Founder mutations may account for the higher prevalence of some subgroups seen in small population studies in countries including Norway (point prevalence of 11.4/100 000 for MC, and 2.5/100 000 for HypoPP) and the Republic of Ireland (prevalence rate 1.69/100 000 for PP)[11,12]. In Ireland, the prevalence of PP was greater than MC (0.32/100 000), likely reflecting a PP founder effect. International, collaborative prevalence studies are required in the future to better understand global differences in genetic variants. Our study provides an up-to-date minimum point prevalence estimate in the largest cohort to date.

Declarations of interest: None

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements:

MGH is supported by an MRC strategic award for International Centre for Genomic Medicine in Neuromuscular Diseases. VV is supported by the HSS for Channelopathies, Mission Possible (National Brain Appeal) and The Jon Moulton Charity. Our work is supported by the UCLH NIHR Biomedical Research Centre. We provide the only NHS England commissioned national diagnostic service and references laboratory for children and adults muscle channelopathies led from University College London NHS Foundation Trust – for further details contact m.hanna@ucl.ac.uk.

References

[1] Vivekanandam V, Munot P, Hanna MG, Matthews E. Skeletal Muscle Channelopathies. Neurol Clin 2020;38:481–91. https://doi.org/10.1016/j.ncl.2020.04.003.

[2] Horga A, Rayan DLR, Matthews E, Sud R, Fialho D, Durran SCM, et al. Prevalence study of genetically defined skeletal muscle channelopathies in England. Neurology 2013. https://doi.org/10.1212/WNL.0b013e31828cf8d0.

[3] Suetterlin K, Matthews E, Sud R, McCall S, Fialho D, Burge J, et al. Translating genetic and functional data into clinical practice: a series of 223 families with myotonia. Brain 2021. https://doi.org/10.1093/brain/awab344.

[4] Vivekanandam V, Männikkö R, Skorupinska I, Germain L, Gray B, Wedderburn S, et al. Andersen–Tawil syndrome: deep phenotyping reveals significant cardiac and neuromuscular morbidity. Brain 2022:1–13. https://doi.org/10.1093/brain/awab445.

[5] Office of National Statistics. Population and household estimates, England and Wales - Office for National Statistics. Census 2021 2022.

[6] Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405–24. https://doi.org/10.1038/gim.2015.30.

[7] Vivekanandam V, Männikkö R, Matthews E, Hanna MG. Improving genetic diagnostics of skeletal muscle channelopathies. Expert Rev Mol Diagn 2020;00:725–36. https://doi.org/10.1080/14737159.2020.1782195.

[8] Matthews E, Manzur AY, Sud R, Muntoni F, Hanna MG. Stridor as a neonatal presentation of skeletal muscle sodium channelopathy. Arch Neurol 2011;68:127–9. https://doi.org/10.1001/archneurol.2010.347.

[9] Thor MG, Vivekanandam V, Sampedro-Castañeda M, Tan SV, Suetterlin K, Sud R, et al. Myotonia in a patient with a mutation in an S4 arginine residue associated with hypokalaemic periodic paralysis and a concomitant synonymous CLCN1 mutation. Sci Rep 2019;9:1–11. https://doi.org/10.1038/s41598-019-54041-0.

[10] Stunnenberg BC, Raaphorst J, Deenen JCW, Links TP, Wilde AA, Verbove DJ, et al. Prevalence and mutation spectrum of skeletal muscle channelopathies in the Netherlands. Neuromuscul Disord 2018;28:402–7. https://doi.org/10.1016/j.nmd.2018.03.006.

[11] Müller KI, Ghelue M Van, Lund I, Jonsrud C, Arntzen KA. The prevalence of hereditary neuromuscular disorders in Northern Norway. Brain Behav 2021;11:1–9.

https://doi.org/10.1002/brb3.1948.

[12] Lefter S, Hardiman O, Ryan AM. A population-based epidemiologic study of adult neuromuscular disease in the Republic of Ireland. Neurology 2017;88:304–13. https://doi.org/10.1212/WNL.0000000000003504.