Acetazolamide efficacy in hypokalemic periodic paralysis and the predictive role of genotype

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ABSTRACT

Objectives: Acetazolamide has been the most commonly used treatment for hypokalemic periodic paralysis since 1968. However, its mechanism of efficacy is not fully understood, and it is not known whether therapy response relates to genotype. We undertook a clinical and genetic study to evaluate the response rate of patients treated with acetazolamide and to investigate possible correlations between response and genotype.

Methods: We identified a total of 74 genotyped patients for this study. These included patients who were referred over a 15-year period to the only UK referral center or to a Chinese center and who underwent extensive clinical evaluation. For all genotyped patients, the response to acetazolamide therapy in terms of attack frequency and severity was documented. Direct DNA sequencing of *CACNA1S* and *SCN4A* was performed.

Results: Only 46% of the total patient cohort (34 of 74) reported benefit from acetazolamide. There was a greater chance of benefit in patients with mutations in *CACNA1S* (31 responded of 55 total) than in those with mutations in *SCN4A* (3 responded of 19 total). Patients with mutations that resulted in amino acids being substituted by glycine in either gene were the least likely to report benefit.

Conclusions: This retrospective study indicates that only approximately 50% of genotyped patients with hypokalemic periodic paralysis respond to acetazolamide. We found evidence supporting a relationship between genotype and treatment response. Prospective randomized controlled trials are required to further evaluate this relationship. Development of alternative therapies is required. *Neurology*® 2011;77:1960-1964

GLOSSARY

hypoPP = hypokalemic periodic paralysis.

Hypokalemic periodic paralysis (hypoPP) is an autosomal dominant neuromuscular disorder characterized by episodes of flaccid skeletal muscle paralysis accompanied by reduced serum potassium levels. It is caused by mutations in 1 of 2 sarcolemmal ion channel genes, *CACNA1S* and *SCN4A*,¹⁻³ that lead to dysfunction of the dihydropyridine receptor or the α subunit of the skeletal muscle voltage-gated sodium channel Nav1.4. Seventy to 80% of cases are caused by mutations of *CACNA1S* and 10% by mutations of *SCN4A*.⁴

Acetazolamide has been the most common treatment choice for hypoPP for nearly 50 years,⁵ although there are no randomized controlled trial data to support its efficacy. Since the discovery of the 2 causative genes, it has been occasionally reported that some patients with *SCN4A* mutations associated with hypoPP reacted adversely to acetazolamide, but other reports have indicated benefit.⁶ However, there have been no larger scale prospective or retrospective studies evaluating possible relationships between genotype and treatment response. In this study, we personally assessed the relationship between acetazolamide treatment response and genotype in

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Table 1 Summary of response to acetazolamide by genotype for cohort 1 (UK cases)

Gene	Mutation	Total no. of genotyped patients treated with ACZ	Beneficial effect	Deleterious effect	No response
CACNA1S	All mutations	14	8	2	4
CACNA1S	R528H	6	4	2	0
CACNA1S	R1239H	7	4	0	3
CACNA1S	R1239G	1	0	0	1

Abbreviation: ACZ = acetazolamide.

18 genotyped cases of hypoPP and compared this to 56 published genotyped cases. These retrospective data indicate that approximately 50% of patients have no useful response to acetazolamide and that genotype is an important factor in treatment response.

METHODS Genetic analysis. Two groups of patients under the authors' personal care were assessed (cohorts 1 and 2). All patients referred to our UK referral center with a possible diagnosis of hypoPP underwent diagnostic genetic testing. We undertook direct automated sequencing of the S4 voltage sensor segments of *CACNA1S* and *SCN4A* (exons 4, 11, 21, and 30 of *CACNA1S* and exons 5, 12, 13, 18, and 24 of *SCN4A*) as described previously.⁴

Standard protocol approvals, registrations, and patient consents. All patients gave written informed consent for the DNA analysis in this study. Ethics committee approval for review of medical records of patients already under our existing care was obtained from the National Hospital for Neurology and Neurosurgery and Institute of Neurology Joint Research Ethics Committee. No intervention was taken beyond the usual clinical care.

Table 2 Summary of response to acetazolamide by genotype for cohort 3 (published cases)

Gene	Mutation	Total no. of genotyped patients treated with ACZ	Beneficial effect	Deleterious effect	No response
CACNA1S	All mutations	39	21	2	16
CACNA1S	R528G	1	0	0	1
CACNA1S	R528H	16	9	0	7
CACNA1S	R1239H	18	11	1	6
CACNA1S	R1239G	3	0	1	2
CACNA1S	R897S	1	1	0	0
SCN4A	All mutations	17	3	11	3
SCN4A	Mutations not specified	4	0	4	0
SCN4A	R669H	2	1	0	1
SCN4A	R672G	4	0	4	0
SCN4A	R672C	1	0	0	1
SCN4A	R672S	5	2	3	0
SCN4A	R1132Q	1	0	0	1

Abbreviation: ACZ = acetazolamide.

Assessment of treatment response in cohorts 1 and 2.

Only those patients in whom a genetic diagnosis of hypoPP had been confirmed and who had been under our follow-up for at least 1 year and treated with acetazolamide were included in the study. Patients underwent full clinical evaluation on a 6-month or once a year basis. A detailed retrospective analysis was undertaken of the clinical records of 14 patients at the UK national reference center for muscle channelopathies neuromuscular clinic (cohort 1). Documented frequency, severity, and duration of paralytic attacks before and after therapy with acetazolamide were analyzed to ascertain response as either beneficial, detrimental, or no change (table 1). A similar survey was undertaken of 4 further patients at a Chinese center in Hangzhou (cohort 2).

Analysis of published cases (cohort 3). We conducted a PubMed search using the key terms hypokalemic periodic paralysis, periodic paralysis, acetazolamide, and carbonic anhydrase inhibitors. Only articles published in English since 1994 (when the genetic basis of hypoPP was identified) were considered. Only cases of genetically confirmed hypoPP were selected (cohort 3). Articles meeting these criteria were reviewed, and any reported treatment and treatment response were recorded (table e-1 on the *Neurology*[®] Web site at www.neurology.org). These data were then used to evaluate the response to acetazolamide by genotype for 56 patients (table 2). Genotyped patients who had received acetazolamide in isolation or with other therapies (spironolactone, potassium supplements, or other) were selected. In an attempt to avoid any bias, the analysis of each of the 3 cohorts was conducted independently by a separate author. A beneficial response was defined as a reduction in frequency, severity, or duration of attacks of paralysis.

RESULTS In cohort 1, 57% of patients with *CACNA1S* mutations, which also represented the cohort as a whole, reported a beneficial response to acetazolamide therapy. The positive response rate increased to 62% when only patients with the most common hypoPP mutations (R528H and R1239H) were considered. Cohort 2 was considered too small to analyze the results in the same way as for the larger cohorts (cohorts 1 and 3), but the overall trend in therapy response was similar; i.e., both the *CACNA1S* Chinese cases (1 R528H and 1 R1239H) but neither of the 2 *SCN4A* cases (2 R672H) benefited from acetazolamide. In cohort 3, 54% of patients with a *CACNA1S* mutation reported a beneficial response compared with only 18% of those with an *SCN4A* mutation. For cohort 3, an overall 43% of patients reported benefit. However, if the R528H and R1239H groups in cohort 3 were considered alone, the benefit improved to 59%.

If the combined results from all 3 cohorts are considered: 31 of 55 (56%) patients with *CACNA1S* mutations benefitted from acetazolamide compared with only 3 of 19 (16%) patients with *SCN4A* mutations ($p < 0.002$ by a χ^2 statistic). Hence, a patient with a *CACNA1S* mutation is 6.9 times as likely to benefit

(A) Diagrammatic representation of Cav1.1 with voltage sensor mutations known to cause hypoPP highlighted. (B) Diagrammatic representation of Nav1.4 with voltage sensor mutations known to cause hypoPP highlighted. *R675G/Q/W has been associated with an atypical phenotype denoted potassium-sensitive normokalaemic periodic paralysis. Modified from reference¹³ with permission from *The Journal of Physiology*.

from acetazolamide therapy than a patient with an *SCN4A* mutation (95% confidence interval 1.6–26.4).

These findings are consistent with previous reports that there is a greater chance of a deleterious effect or no beneficial effect from acetazolamide in patients with hypoPP due to *SCN4A* mutations but that this does not apply to all such patients. Further evaluation of the relationship between precise genotype and treatment response was undertaken using the larger cohorts 1 and 3. We observed in both cohorts that when one of the arginine residues located at the extracellular side of the sarcolemma (figure) was substituted for a glycine residue (R528G, R1239G, or R672G), none of these patients (0 of 9) reported a beneficial response to acetazolamide, and there was often a deleterious effect although the number of patients with these substitutions was relatively small.

Substitutions of the more inwardly placed arginine at position R675 in *SCN4A* have been described. Patients with these substitutions (R675G/Q/W) who received acetazolamide therapy were identified in each cohort but were not included in the final analysis because the phenotype has been reported as potassium-sensitive normokalemic periodic paralysis and not as hypoPP.⁷

DISCUSSION There are no consensus guidelines for the treatment of hypoPP. Current pharmacologic agents commonly used include potassium supplements, potassium-sparing diuretics, and carbonic anhydrase inhibitors (acetazolamide and dichlorphenamide). Dichlorphenamide is the only therapy for hypoPP to have undergone a randomized double-blind placebo-controlled crossover trial. This trial showed significant efficacy of dichlorphenamide in reducing attack frequency, but the inclusion criteria were based on a clinical diagnosis of hypoPP and not genetic confirmation.^{8,9} A second randomized controlled trial of the efficacy of dichlorphenamide in genotyped cases of both hypokalemic and hyperkalemic periodic paralysis is currently open (see www.clinicaltrials.gov). Aside from these trials, there is very little trial evidence to support the use of any treatment in hypoPP and no randomized controlled trial evidence supporting the most common choice, acetazolamide.

The data presented here suggest that at least half the patients treated with acetazolamide do not get a satisfactory response. A tentative correlation between detrimental response and genotype does emerge, and those patients with substitutions to glycine of arginine residues situated toward the extracellular side of the voltage

sensors of either Cav1.1 or Nav1.4 (figure) may be predicted not to respond to acetazolamide. This observation is particularly noteworthy in light of experimental data indicating that the deleterious effects of the R672G substitution on channel gating are insensitive to reductions in pH in vitro, which is in contrast to the deleterious effects of the R669H mutation that were ameliorated by an acidic pH. These in vitro observations predict that such patients would not respond to acetazolamide, which produces a metabolic acidosis.¹⁰ The deleterious effects studied relate to the ion-selective α pore. More recent studies have identified an anomalous proton-selective gating pore due to R669H and R672H *SCN4A* mutations and a less selective cation-conducting pore in the R672G *SCN4A* mutation.^{11,12} Similar gating pores have yet to be identified in the *CACNA1S* mutations but are proposed as a likely pathomechanism. An additional suggestion is that the R to H substitutions are likely to have a greater effect on pH concentration gradients, and, as a result, there is more likelihood of a beneficial response to acetazolamide therapy than with the R to G substitutions. Overall, these data support the view that it is important to achieve a genetic diagnosis in each patient to help guide treatment.

Without randomized controlled trials, the exact efficacy of acetazolamide is unknown. In addition, its mechanism of action is unclear,¹³ although it has become first-line therapy and has been suggested to have benefit for the majority of patients for almost half a century.¹⁴ Our data suggest, however, that the response rate may be more modest than generally considered at present.

This retrospective clinical and literature evaluation collates the single largest evaluation of genotyped hypoPP patients reported. Our observations indicate that acetazolamide has a benefit for at most only 50% of patients. However, we observed that the response rate improves to 60% if only those patients with common *CACNA1S* mutations are considered. Furthermore, there is a suggestion that patients with arginine substitutions to glycine in the residues of the voltage sensor near the extracellular side of the sarcolemma may be predicted to respond poorly, and this is supported by experimental observations.

Conclusive evidence for a unified approach to the treatment of hypoPP is lacking, and retrospective data as outlined here have limitations. However, the data reported here support the view that randomized controlled trials of available therapies are required. Furthermore, given that the response rate is on the order of 50%, we suggest that new therapies need to be developed for patients with hypoPP.

AUTHOR CONTRIBUTIONS

Dr. Ke, Dr. Sud, A. Haworth, and Dr. Davis contributed to the analysis and interpretation of data. Dr. Matthews and Dr. Portaro contributed to the study design, analysis and interpretation of data, and writing of the manuscript. Dr. Griggs and Dr. Hanna contributed to study design and the writing/revision of the manuscript.

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DISCLOSURE

Dr. Matthews, Dr. Portaro, Dr. Ke, Dr. Sud, A. Haworth, and Dr. Davis report no disclosures. Dr. Griggs serves as Chair of the Executive Committee of the Muscle Study Group, which receives support from pharmaceutical companies; has served on scientific advisory boards for The National Hospital Queen Square and PTC Therapeutics, Inc.; serves on the editorial boards of *NeuroTherapeutics* and *Current Treatment Opinions in Neurology* and as Correspondence Editor for *Neurology*[®]; is immediate Past President of the American Academy of Neurology; receives royalties from the publication of *Andreoli and Carpenter's Cecil Essentials of Medicine, 8th edition* (W.B. Saunders Company, 2000, 2004, 2007, and 2010) and *Cecil Textbook of Medicine, 24th edition* (Saunders, 2000, 2004, 2008, and 2010, in press); and has received research support from Taro Pharmaceuticals and the NIH/NINDS, the FDA, and the Muscular Dystrophy Association. Prof. Hanna serves as Deputy Editor for the *Journal of Neurology, Neurosurgery & Psychiatry*.

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Classification scheme requirements for therapeutic questions

Class I. A randomized, controlled clinical trial of the intervention of interest with masked or objective outcome assessment, in a representative population. Relevant baseline characteristics are presented and substantially equivalent among treatment groups or there is appropriate statistical adjustment for differences.

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Class III. All other controlled trials (including well-defined natural history controls or patients serving as their own controls) in a representative population, where outcome is independently assessed, or independently derived by objective outcome measurements.

Class IV. Studies not meeting Class I, II, or III criteria including consensus or expert opinion.

AAN classification of recommendations

A = Established as effective, ineffective, or harmful (or established as useful/predictive or not useful/predictive) for the given condition in the specified population. (Level A rating requires at least two consistent Class I studies.)

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C = Possibly effective, ineffective, or harmful (or possibly useful/predictive or not useful/predictive) for the given condition in the specified population. (Level C rating requires at least one Class II study or two consistent Class III studies.)

U = Data inadequate or conflicting; given current knowledge, treatment (test, predictor) is unproven.