Periodic paralysis mutation MiRP2-R83H in controls

Interpretations and general recommendation

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Abstract—An R83H point mutation in KCNE3-encoded MiRP2 has been reported to cause 2% of all cases of familial periodic paralysis. The authors found MiRP2-R83H in 3 of 321 control subjects and in 5 unaffected related individuals. Provocation of an unaffected carrier with glucose or KCl did not induce weakness. The authors propose that causality criteria for mutations require exclusion of mutations in $n = \ln(P)/\ln(1 - p_1)$ ethnically matched control chromosomes (P = acceptable error probability; p_1 = mutation prevalence in patient chromosomes).

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Familial dyskalemic periodic paralysis (FPP) is an autosomal dominant muscle disorder with a combined prevalence of 1:50,000.[1] It is characterized by episodes of flaccid weakness discriminated according to the change in serum potassium during the weakness: hypokalemic (HypoPP) and hyperkalemic (HyperPP) FPP. Oral administration of potassium triggers attacks, and glucose is a remedy for HyperPP, whereas glucose provokes attacks of HypoPP, which are relieved by potassium intake.[2]

An R83H point mutation in the MiRP2 protein, a potassium channel subunit encoded by *KCNE3*, has been described to cause HypoPP and HyperPP in a pedigree consisting of three and two blood relatives, respectively.[3] MiRP2-R83H was identified later in a third phenotype, thyrotoxic PP (TPP), in an individual with two unaffected mutation-carrying children.[4] In contrast, another study identified the mutation in 8 of 506 control subjects and in a genetically clarified HypoPP family, in which MiRP2-R83H did not cosegregate.[5] To check these findings, we tested for MiRP2 mutations in a large control sample and in patients with HypoPP, HyperPP, TPP, or paramyotonia congenita (PC), a muscle disorder allelic to HyperPP and HypoPP but clinically distinguishable by muscle stiffness worsening with exercise and potential muscle weakness triggered by cold and potassium.

Patients and methods. Experiments were approved by the Ethics Committee of Ulm University and done in concordance with the Declaration of Helsinki. Blood samples were taken from 528 individuals with their informed consent: 62 with HypoPP, 76 with HyperPP, 61 with PC, 8 with TPP, and 321 normal ethnically matched control subjects. DNA was extracted, and two PCR reactions were performed on each sample to cover the coding region of the single exon of *KCNE3* (primers GTTTGAGCTTCTACCGAG and TGTAGGAGTTGTCATCAC, yielding a 220-base pair [bp] product at 55 °C, and CAACCAGACTGAAGAGAG and CAGTCCACAGCAGAGTTC, yielding a 271-bp product at 55 °C). After agarose gel band purification, PCR products were sequenced. Subsequent PCR for reverse sequencing was performed of all samples showing a base exchange for verification. For testing of I1160V in the sodium channel Nav1.4, exon 19 of the *SCN4A* gene was amplified using GGAGGCACTGGCAATGGAC and AGGGTGGTGGGTCACACTCA, yielding a 334-bp product at 59 °C, which also was sequenced.

Provocative clinical testing for PC and FPP was performed with informed proband consent. Three patients were tested to confirm the diagnosis. Three individuals without FPP who were originally referred for differential diagnosis of an episodic attack were used as control subjects. For PC testing, patients performed bicycle exercise for 30 minutes at 50 W, ingested 1.3 mmol/kg body weight KCl, and had their forearm cooled for 30 minutes at 15 °C; for HyperPP, KCl provocation was performed as above; and for HypoPP, ingestion of 2 g/kg body weight glucose was performed as described previously.[6] Strength was measured during a 5-hour period using a grip strength force transducer. Minimal strength values were indicated relative to starting force.

Results. We detected MiRP2-R83H in the following unrelated individuals: 3 of 321 control subjects, 0 of 62 patients with HypoPP, 1 of 76 patients with HyperPP, and 0 of 8 patients with TPP. The patient with HyperPP had a negative family history. The three normal control subjects with MiRP2-R83H did not have signs of muscle weakness, and family history was negative. All three were men, aged 46 to 65 years, who were referred to hospital for hernias. No other base changes in *KCNE3* coding for amino acid changes in MiRP2 were detected in any sample.

In 61 unrelated patients with PC, we detected MiRP2-R83H once. In the corresponding pedigree, the PC phenotype did not cosegregate. Instead, Nav1.4-I1160V segregated without recombinants (figure 1). Therefore, MiRP2-R83H does not cause PC. However, because Nav1.4-I1160V had been described to cause acetazolamide-

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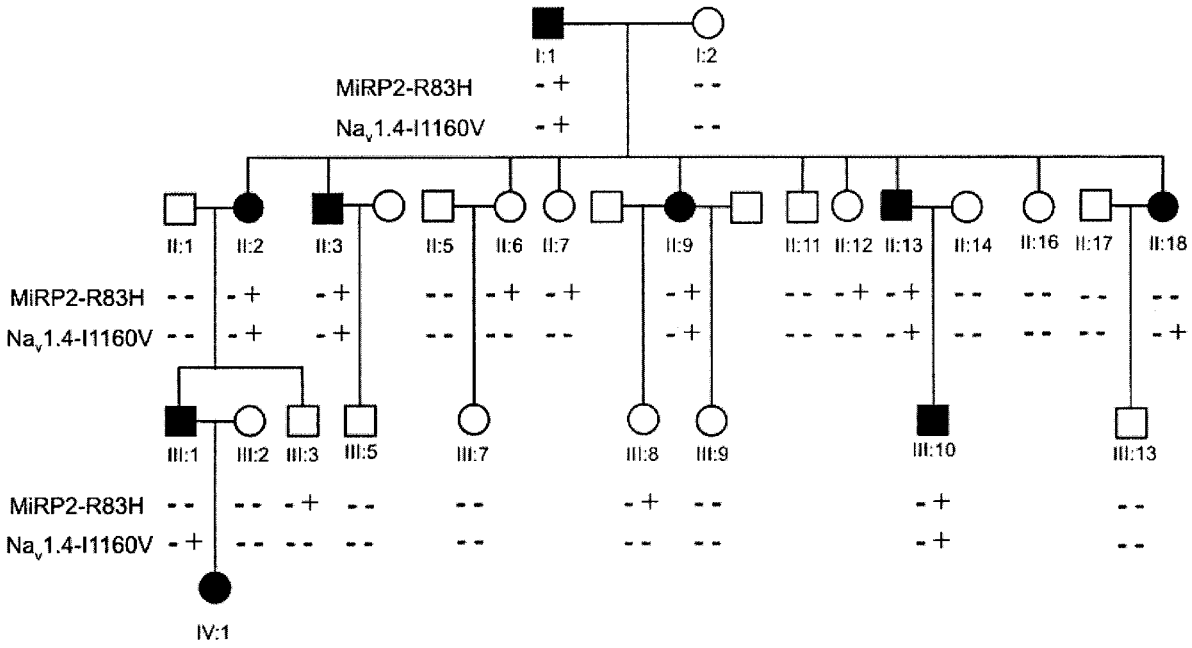

Figure 1. Arbitrary occurrence of R83H in a family with paramyotonia congenita (PC). The authors examined all family members. Affected individuals: closed; unaffected: open; males: square; females: circular symbols. Presence of mutations is indicated by +. The known Nav1.4-I1160V mutation in the sodium channel segregates with the clinical status, whereas MiRP2-R83H does not. Individuals II:6, II:7, II;12, III:3, and III:8 had no muscle symptoms. Comparison of the response with PC-provocative tests in Individuals II:18 and II:9 does not support an MiRP2-R83H contribution to the phenotype. Provocation for hypokalemic familial periodic paralysis (FPP) and hyperkalemic FPP did not elicit weakness in Individual III:8.

responsive myotonia (stiffness without weakness phenotype with features overlapping PC) in the only published pedigree, we performed provocative testing for PC on Individual II:18, carrying only I1160V but not R83H.[7] The bicycle test reduced strength to 55%, forearm cooling to 50%, and KCl loading to 70%. This clearly confirmed the PC diagnosis because otherwise there would have been force reduction of <10%. Next, we tested a carrier of MiRP2-R83H and Nav1.4-I1160V, Individual II:9. She showed strength reduction to 60%, 60%, and 80% in the same tests, respectively, not supporting an aggravation of the weakness component of the PC phenotype by MiRP2-R83H.

We re-examined the five R83H carriers without I1160V—Individuals II:6, II:7, II:12, III:3, and III:8—for possible effects of MiRP2-R83H; none showed episodic weakness. We then provoked one of these, Individual III:8, with glucose and on the following day with KCl. The time courses of muscle strength in both tests did not differ from those of three control individuals without R83H. From this, we conclude that R83H cannot reproducibly generate symptoms on provocation.

Discussion. Table 1 gives an overview of the MiRP2-R83H studies. A possible explanation for the discrepant results is reduced penetrance of the mutation. Assuming there is no difference between the population screened in the different studies, the penetrance can be calculated most accurately by combining all data: the portion of 4 of 342 = 1.17% of MiRP2-R83H carriers with FPP, the frequency of 1:50,000 = 0.002% of patients with FPP in the general population, and the frequency of 11 of 1,047 = 1.05% for MiRP2-R83H carriers in the general popu-

Table *Summary of the MiRP2-R83H studies*

Study	FPP	TPP	PC	Controls	Sum
Ref 3	2/100	—	—	0/120	2/220 = 0.91%
Ref 4	—	1/15	—	0/105	1/120 = 0.83%
Ref 5	1/104	—	—	8/506	9/610 = 1.48%
Our study	1/138	0/8	1/61	3/321	5/528 = 0.95%
Sum	4/342 = 1.17%	1/23	1/61	11/1052 = 1.05%	17/1478 = 1.15%

The number of mutation carriers and the number of individuals tested for each group are indicated. Note that the sums in the rightmost column all yield similar values suggesting that the mutation prevalence is independent of the disorders studied.

FPP = familial periodic paralysis; TPP = thyrotoxic periodic paralysis; PC = paramymotonia congenita.

lation.[1] Penetrance is the quotient of FPP mutation carriers (1.17% × 0.002%) and mutation carriers in the general population (1.05%). Therefore, penetrance = 1.17% × 0.002%/1.05% = 0.0022% or 1:44,870, which means that of 44,871 mutation carriers, only one actually develops the disease. This penetrance is not significantly different from the disease prevalence (1:50,000) and would contradict a direct causal relationship between MiRP2-R83H and FPP.

MiRP2-R83H is not recessive because no additional MiRP2 mutations were found in the first report nor in our study.[3] Another possibility is a systematic difference between the populations in the studies that affects the disease-causing potency of the mutation (i.e., ethnic origin, sex, concomitant disorders such as thyrotoxicosis, additional mutations in other genes, and environmental factors like food or climate). For the original report, the ethnic group is unknown; all affected mutation carriers were men; and thyrotoxicosis is unlikely because patients were diagnosed with primary FPP instead of TPP.[3] In our study, the mutation carriers were white, of both sexes, and without thyrotoxic signs. Clarification of the importance of the other factors may merit further study.

A last possibility suggests MiRP2-R83H does not contribute to FPP, and the mutation would have been identified in control subjects of the first report if a larger number had been studied.[3] Generally, a method to determine the required number of control subjects depending on the mutation prevalence in patient chromosomes, p_1, is warranted. Let the frequency in control chromosomes be p_0; the probability that an arbitrary control chromosome does not carry the mutation then would be $1 - p_0$. Because the world control population is large, the probability P of arbitrarily choosing n chromosomes thereof without the mutation can approximated by $P = (1 - p_0)^n$. The null hypothesis would be that the mutation frequency is equal in patient and control chromosomes, $p_0 = p_1$. The error of falsely excluding the null hypothesis equals the probability of arbitrarily having chosen n control chromosomes without the mutation (i.e., exactly P; figure 2A). Vice versa, the number of required control chromosomes, n, can be estimated when tolerating a maximal error probability of P: $P = (1 - p_0)^n$ or $P = (1 - p_1)^n \Leftrightarrow \log_{(1 - p1)}(P) = n \Leftrightarrow n = \ln(P)/\ln(1 - p_1)$ (figure 2B). It would be advisable to tolerate no more than a 1% maximal error and thus test $n = -4.6/\ln(1 - p_1)$ ethnically matched control subjects.

In the first report, MiRP2-R83H was found in 2% of patients (1% of patient chromosomes), indicating that the error probability was $P = (1 - 0.01)^{120} = 8.9\%$, falsely concluding that the mutation is not present in control subjects (see figure 2A).[3] We recommend screening $n = -4.6/\ln(1 - 0.01) = 460$ control chromosomes or 230 samples, almost twice as many as the 120 tested (see figure 2B). Clearly, the error probability does not establish diagnosis, but diagnosis may be achieved in this case by examining which other factors contribute to the phenotype.

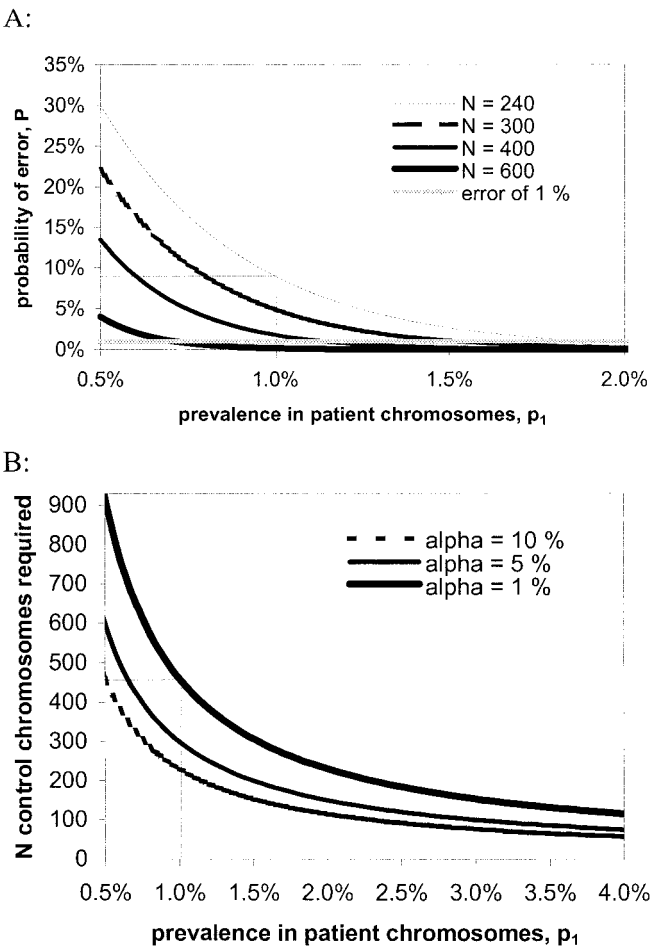

Figure 2. (A) Error probability for falsely excluding the null hypothesis. If p_0 is the mutation prevalence in control chromosomes, the probability for n control chromosomes not to carry the mutation can be approximated by $(1 - p_0)^n$. If p_1 is the mutation prevalence in patient chromosomes, the error probability of falsely excluding the null hypothesis ($p_0 = p_1$) therefore is $P = (1 - p_1)^n$. P is plotted as a function of p_1 for different n. P for n = 240 chromosomes and p_1 = 1% is indicated, 8.9%. (B) Proposed number of control chromosomes depending on mutation prevalence in patient chromosomes. The equation $P = (1 - p_1)^n$ is solved to obtain $n = \ln(P)/\ln(1 - p_1)$ as the proposed number of control chromosomes n for testing mutation causality. For the example, n is indicated for p_1 = 1%, n = 460.

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