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Mutation in DHP receptor $\alpha 1$ subunit (CACLN1A3) gene in a Dutch family with hypokalaemic periodic paralysis

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Abstract

Hypokalaemic periodic paralysis (HypoPP) is characterised by transient attacks of muscle weakness of varying duration and severity accompanied by a drop in serum potassium concentration during the attacks. The largest known HypoPP family is of Dutch origin and consists of 277 members in the last five generations, 55 of whom have HypoPP inherited in an autosomal dominant pattern. Forty-eight persons including 28 patients with a proven diagnosis of HypoPP were used for linkage analysis. Microsatellite markers were used to exclude 45 to 50% of the genome and linkage to chromosome 1q31-32 was found. No recombinants were found between HypoPP and D1S412 and a microsatellite contained within the DHP receptor $\alpha 1$ subunit (CACLN1A3) gene. A previously reported G to A mutation causing an arginine to histidine substitution at residue 528 in the transmembrane segment IIS4 of the CACLN1A3 gene was shown in patients by restriction analysis of genomic PCR products.

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Hypokalaemic periodic paralysis (HypoPP) belongs to the group of familial periodic paralyses. These diseases are characterised by transient attacks of muscle weakness of varying duration and severity.¹ Changes in serum potassium concentration during the attacks constitute the basis for subdividing periodic paralysis into hypo-, hyper-, and normokalaemic forms.¹

The genetic locus of the normokalaemic form is unknown. Hyperkalaemic periodic paralysis (HYPP) is characterised by attacks of flaccid muscular weakness as well as myotonia, which may even be the dominant symptom. Myotonia and paralysis may also occur in separate persons within the same family.² This association has now been solved at the genetic level: HYPP both with and without myotonia or para-

myotonia,^{3,4} paramyotonia congenita,^{4,5} and atypical myotonia congenita⁶ are all known to result from mutations at various sites in the gene coding for the α unit of the adult isoform of the skeletal muscle sodium channel (SCN4A) on chromosome 17q.^{3,6,7}

HypoPP (MIM 170400) is the most frequent form of periodic paralysis. Although it is usually transmitted as an autosomal dominant disease, sporadic cases do occur.^{1,8,9} The initial attacks typically occur during the first two decades of life, sometimes increasing in frequency to weekly or even daily occurrences, but decreasing in frequency over the age of 30.¹⁰ Permanent muscular weakness on the basis of a vacuolar myopathy develops in some people and may lead to severe disability in older patients.^{10,11} The pathophysiology of HypoPP has not been elucidated. An increased transmembrane conductance of Na⁺,^{12,13} increased activity of the sodium potassium pump,¹⁴ and faults in K⁺ conductance^{15,16} have been proposed as the basic defect. However, even detailed neurophysiological in vitro investigations of muscle fibres have not provided an adequate explanation.¹⁴

We were investigating the largest known HypoPP family^{10,11} for linkage when the assignment of the HypoPP locus to chromosome 1q31-32 was published.¹⁷ The data of Fontaine *et al*¹⁷ suggest genetic homogeneity and the DHP receptor (calcium channel) alpha 1 subunit (CACLN1A3) as a candidate gene and recent studies have indeed shown mutations in this gene.^{18,19} In the present study we confirmed linkage to 1q31-32 and were able to pinpoint a specific mutation in the CACLN1A3 gene.

Material and methods

PEDIGREE

The family has 277 members in the last five generations, 55 of whom have HypoPP.^{10,11,20} Forty-five affected persons were alive at the start of the investigations and 33 were personally investigated by one of the authors (TPL). Nineteen patients had typical attacks,

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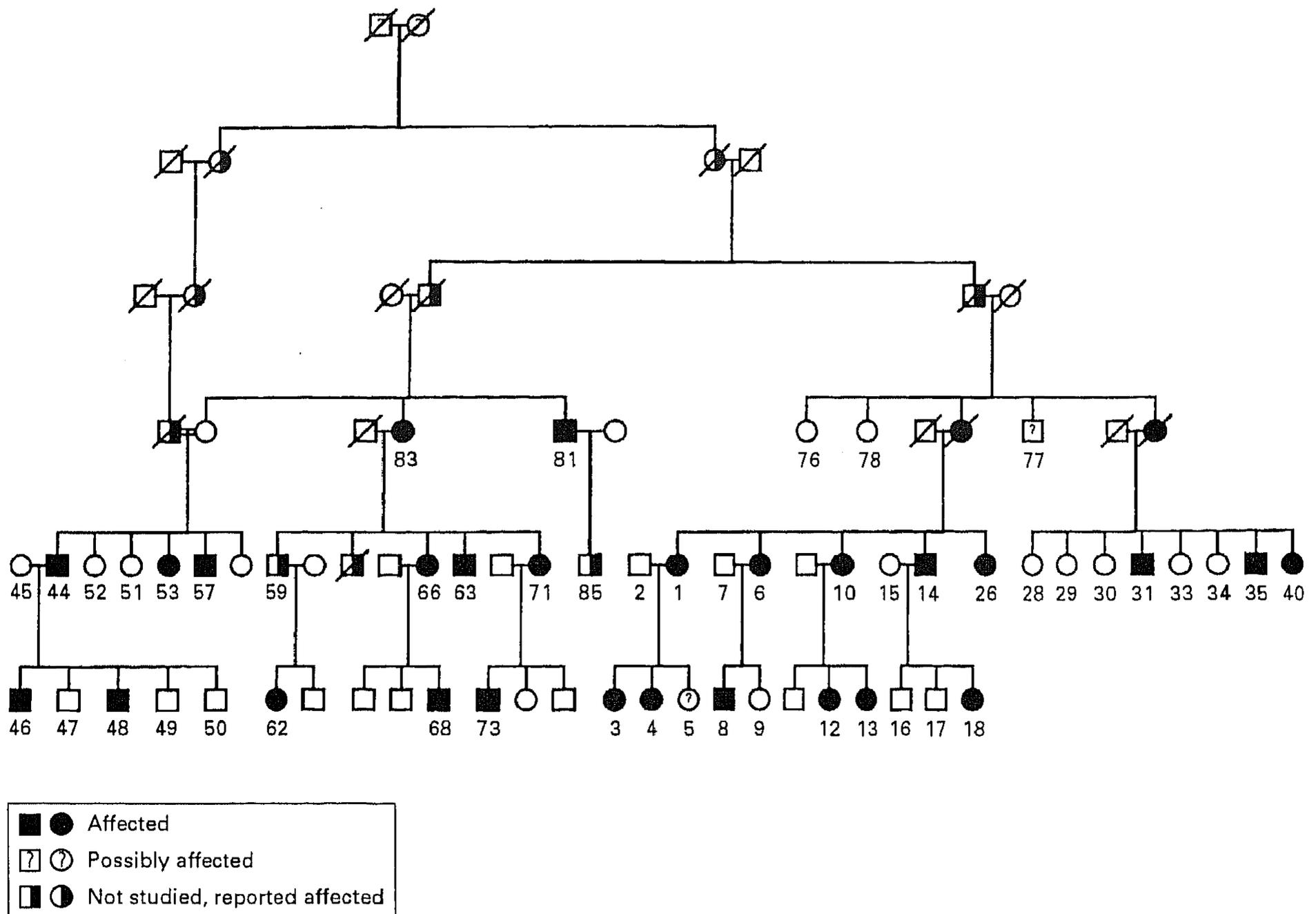


Figure 1 The part of the Dutch HypoPP family used for linkage analysis. For a complete pedigree see Links *et al.*^{10,11}

while 14 had muscle weakness without attacks.¹¹ The diagnosis of HypoPP was based on clinical history, neurological examination, and a reduced muscle fibre conduction velocity.²⁰ The diagnosis was based on the clinical history and notes from other hospitals in the remaining cases and in dead patients.^{10,11} The mean age of onset of attacks was 15.6 (range 11–19) in men and 14.9 (range 9–18) in women. Forty-eight persons including 28 patients, all over the age of 20, were used for linkage analysis (fig 1). An autosomal dominant mode of inheritance was noted in the family. If the criteria mentioned above are used for diagnosis, the disease has complete penetrance.^{10,11}

DNA ANALYSIS

Blood samples were collected from 48 persons and genomic DNA was isolated by phenol-chloroform extraction.²¹ Typing of microsatellite markers was performed as previously described.^{22,23} Microsatellite markers from the Dutch Microsatellite Marker Collection²³ were used for the random gene search. For confirmation of linkage to chromosome 1q31–32, the following markers were used: D1S158, D1S53, D1S412, D1S413, D1S249, and CACNL1A3^{24,25}; primer sequences are available through the Human Genome Data Base. PCR reactions were done on 50 ng of template DNA with $\alpha^{32}\text{P}$ -CTP in a volume of 15 μl in 96 well microplates as previously

described²³ except that end labelling of one of the primers was used for the CACNL1A3 marker. After electrophoresis, the gels were dried and exposed overnight to x ray film (Kodak X-AR).

LINKAGE ANALYSIS

Two point and multipoint lod scores were computed with the LINKAGE program package version 5.10.^{26,27} HypoPP was considered to be autosomal dominant with a penetrance of 100%. The frequency of the gene in the general population was estimated as 0.0001.¹⁷

MUTATION SCREENING

The G to A mutation in codon 528 of the CACNL1A3 gene causes the loss of a *Bbv*I restriction site.¹⁹ Genomic DNA was amplified by PCR with the forward primer 5'-GGA-GATCCTGCTGGTGGAGTCG-3' and the reverse primer 5'-TCCTCAGGAGGCGGATGCAG-3' according to the protocol of Jurkat-Rott *et al.*¹⁹ After digestion with *Bbv*I, the PCR products were run on a 15% polyacrylamide gel. Normal controls show two bands of 44 bp and 33 bp; mutants show an additional band of 77 bp, representing the undigestible PCR product.

Results

At first, candidate regions containing ion channel or related genes were examined to exclude

Pairwise lod scores between chromosome 1q markers and HypoPP

Locus	Recombination fraction							Z _{max}	θ _{max}
	0.00	0.01	0.05	0.10	0.20	0.30	0.40		
D1S158	-9.10	-3.89	-1.87	-0.80	0.21	0.44	0.29	0.44	0.30
D1S412	6.07	5.99	5.63	5.12	3.96	2.63	1.19	6.07	0.00
D1S413	0.33	0.67	1.47	1.58	1.22	0.68	0.22	1.59	0.09
D1S53	-4.93	-1.74	-0.46	-0.00	0.26	0.22	0.08	—	—
CACNL1A3	3.24	3.17	2.88	2.50	1.73	1.01	0.42	3.24	0.00

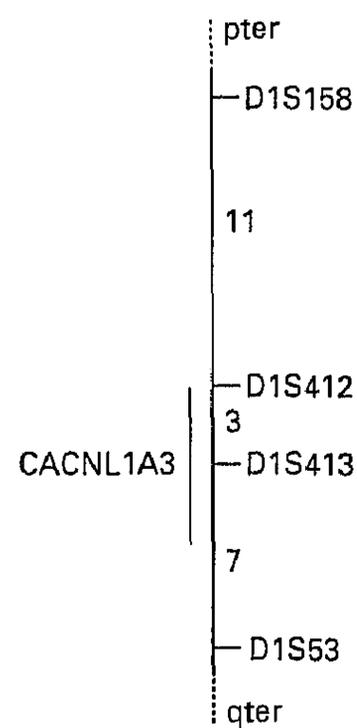


Figure 2 Genetic regional map of chromosome 1q. Markers are indicated with their respective genetic distances; positions obtained through the Human Genome Data Base.

45 to 50% of the genome. Subsequent to the linkage finding of Fontaine *et al.*¹⁷ markers detecting the loci D1S412, D1S413, and CACNL1A3 (fig 2) were analysed in our family, resulting in convincing lod scores indicative of linkage. The maximum two point lod score (Z_{max}) between HypoPP and the chromosome 1q loci was 6.07 at a recombination fraction (θ) of 0.00 from D1S412. The calcium channel CACNL1A3 was also linked to HypoPP: Z_{max} = 3.24 at θ_{max} = 0.00 (table). Multipoint analysis confirmed that the most likely location of the HypoPP gene is close to locus D1S412 (fig 3). Haplotyping showed no recombinants between the HypoPP locus and markers D1S412, D1S413, and CACNL1A3.

PCR amplification of genomic DNA was performed to screen for the recently found G to A mutation in codon 528 of the CACNL1A3 gene, causing loss of a *Bbv*I restriction site. All patients showed an additional 77 bp fragment of undigested amplification product (not shown), confirming the presence of this mutation in this Dutch HypoPP family.

Discussion

Because this is the largest known family with HypoPP, our finding of linkage of HypoPP to chromosome 1q31-32 strongly supports the data of Fontaine *et al.*¹⁷ The penetrance of the gene defect appears to be complete, although the age of onset varies and the clinical manifestation may be permanent and progressive muscle weakness instead of paralytic attacks.^{10,11}

Haplotype analysis showed several recombinants between HypoPP and D1S158 and D1S53, but not with D1S412 and the microsatellite located within the CACNL1A3 gene. Recently Jurkat-Rott *et al.*¹⁹ found a G to A mutation in the transmembrane segment IIS4 in several independent HypoPP patients. This mutation was also found in our Dutch HypoPP family.

This particular calcium channel is an oligomeric protein composed of two high molecular weight polypeptide subunits (α 1 and 2) and three smaller β, γ, and δ units.^{28,29} The α 1 units form the ion pore structure and also function as voltage sensors.^{30,31} The gene is mutated in the muscular dysgenesis mouse (*mdg*), a lethal autosomal recessive disorder in which there is a total lack of excitation-contraction coupling in homozygotes.³²

It is rather surprising to find a calcium channel gene defect implicated in the pathophysiology of HypoPP. As referred to above, most investigators have favoured other defects to explain the hypokalaemic attacks. Nevertheless, early suggestions of disturbed Ca²⁺ release to the contractile elements³³ and an enhanced deposition of Ca²⁺ in the sarcoplasmic reticulum³⁴ already suggested an inadequate release of calcium as the underlying defect in HypoPP.^{10,11} Furthermore, the skeletal muscle calcium channels do play a key role in the excitation-contraction coupling.²⁶ These findings might contribute to the understanding of the occurrence of paralysis in HypoPP. Further studies need to be performed in order to elucidate the relationship between the calcium channel defect and the drop in serum K⁺ concentration and the pathogenesis of permanent progressive muscle weakness.

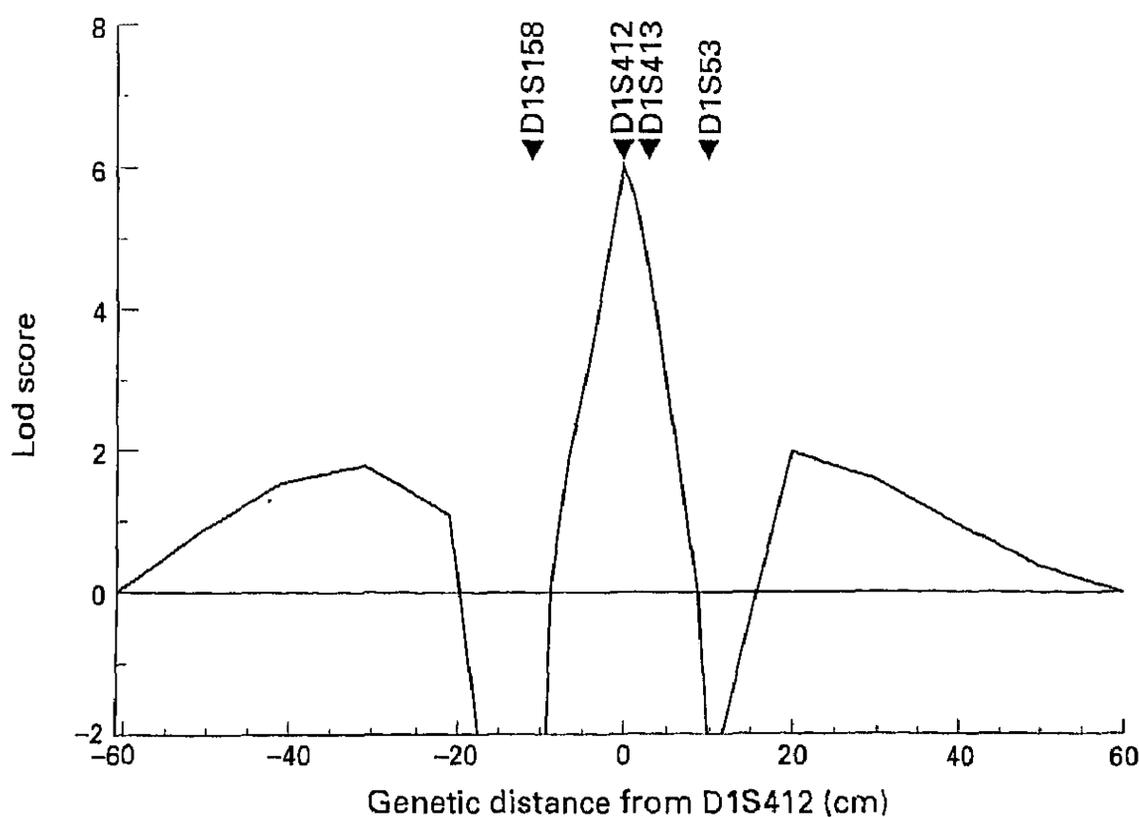


Figure 3 Multipoint analysis. Multipoint lod scores for the HypoPP locus with respect to chromosome 1q markers are shown.

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