European Journal of Human Genetics (2004) 12, 1073–1076

www.nature.com/ejhg

SHORT REPORT

A second locus mapping to 2q35–36 for familial pseudohyperkalaemia

Massimo Carella[1,8], Adamo Pio d'Adamo[1,8], Sabine Grootenboer-Mignot[2,3], Marie C Vantyghem[4], Laura Esposito[1], Angela D'Eustacchio[1], Romina Ficarella[1], Gordon W Stewart[5], Paolo Gasparini[1,6], Jean Delaunay[2,3] and Achille Iolascon*[,7]

[1]*TIGEM, Telethon Institute of Genetics and Medicine, Naples, Italy;* [2]*Laboratoire d'Hématologie, d'Immunologie et de Cytogénétique, Hôpital de Bicêtre, AP-HP, Faculté de Médecine Paris-Sud, France;* [3]*INSERM U 473, Le Kremlin-Bicêtre, France;* [4]*Clinique Marc-Linquette, Lille, France;* [5]*Rayne Institute, University Street, London, UK;* [6]*Medical Genetics, Second University of Naples, Naples, Italy;* [7]*Department of Biochemistry and Medical Biotechnologies – CEINGE, Federico II University, Naples, Italy*

Familial pseudohyperkalaemia (FP) is a symptomless, dominantly inherited red cell trait, which shows a 'passive leak' of K^+ cations into the plasma upon storage of blood at room temperature (or below). There are no haematological abnormalities. The loss of K^+ is due to a change in the temperature dependence of the leak. The Scottish case initially described, FP Edinburgh, maps to 16q23-qter. Here we studied a large kindred of Flemish descent with FP, termed FP Lille, which was phenotypically identical to the Edinburgh FP. In FP Lille, however, the responsible locus mapped to 2q35–36, with a Lod score of 8.46 for marker D2S1338. We infer that FP Edinburgh and FP Lille, although they are phenocopies of one another, stem from two distinct loci, *FP1* (16q23-qter) and *FP2* (2q35–36), respectively. This duality hints at the possibility that the protein mediating the leak might be a heterodimer. No mutation was found in three plausibly candidate genes: the *KCNE4* gene, the *TUBA1* gene and a predicted gene located in genomic contig NT_005403.

European Journal of Human Genetics (2004) **12,** 1073–1076. doi:10.1038/sj.ejhg.5201280
Published online 6 October 2004

Keywords: pseudohyperkalaemia; 2q35–36; erythrocytes

Introduction

Familial pseudohyperkalaemia (FP) is a dominantly inherited genetic trait, in which a temperature-dependent, *in vitro*, loss of K^+ cations from red cells is associated with normal haematology.[1] The basic physiopathological abnormality lies in the temperature dependence of the 'passive leak' to K^+ across the red cell membrane. FP 'Edinburgh' was the original case discovered.[1] These cases can be diagnosed by measuring K^+ movements across the red cell membrane as a function of temperature. Typically, heparinised red cells will lose abnormal amounts of K^+ at both 20 and 0°C due to an abnormality in the temperature dependence of the so-called 'passive leak' to K^+. This abnormality in temperature dependence of the leak can be confirmed using isotopic tracer techniques.[2,3] The responsible gene maps to 16q23-qter.[2]. We present here a large French family of Flemish descent with FP, FP 'Lille'. The cation fluxes were indistinguishable from those in FP Edinburgh. However, microsatellite analysis excluded the 16q23-qter locus. A genome scan allowed to map FP 'Lille' to 2q35–36 with a Lod score of 8.46 for marker D2S1338. Thus, FP Edinburgh and FP Lille, although they are phenocopies of one another, stem from locus *FP1*

***Correspondence: Dr A Iolascon, Medical Genetics, Department of Biochemistry and Medical Biotechnologies, CEINGE – Advanced Biotechnologies, Via Comunale Margherita 482, 80100, Naples, Italy.**
Tel: +39 081 37 22 897; Fax: +39 081 37 22 808;
E-mail: iolascon@dbbm.unina.it
[8]These two authors contributed equally to the work
Received 27 February 2004; revised 1 July 2004; accepted 6 July 2004

(16q23-qter) or *FP2* (2q35–36), respectively. This duality may suggest that the protein mediating the leak is a heterodimer. We sequenced three plausibly candidate genes: (i) the *KCNE4* gene, encoding a Isk-related, potassium voltage-gated channel, (ii) the *TUBA1* gene, encoding tubulin $\alpha 1$ and the (iii) predicted gene encoding an ABC transporter and located in genomic contig NT_005403. None displayed apparent mutations.

Methods

The family has previously been described.[4] After informed consent from each subject, we studied 34 members, of which 23 were carrying the trait. The *in vitro* increase of plasma $[K^+]$, the fluxes of K^+ across the membrane and the dependence of the leak–temperature curve were studied as previously described (Grootenboer *et al*[5], and references therein) (Figure 1). Members with kalaemia ≥ 7 mmol/l after 6 h of incubation at 9°C were considered as carrying the FP trait. Those with kalaemia ≤ 6 mmol/l under the same conditions were considered as not carrying the trait. Four members were not included in the gene scan because the increase of kalaemia was ambiguous (III.8 and III.18), or technical problems had occurred (II.10 (no DNA spared) and III.12 (haemolysis)).

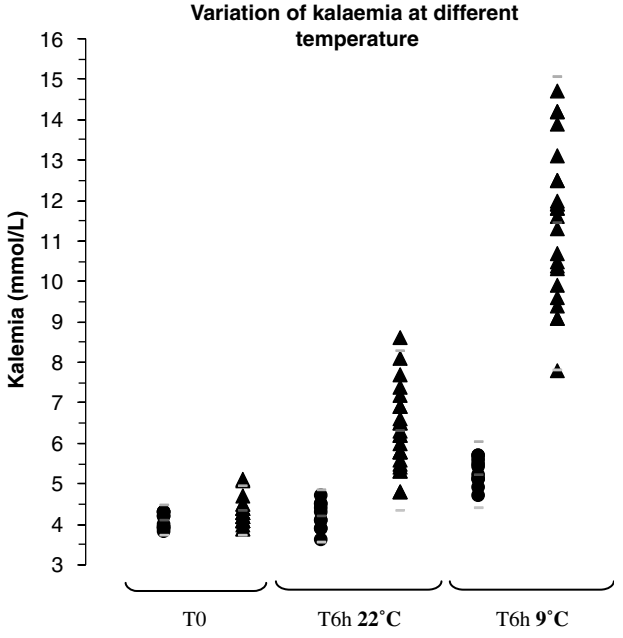

Figure 1 The *in vitro* increase of kalaemia at 22 and 9°C. At 22°C blood incubation (6 h), kalaemia raised moderately in patients with FP2 ($m \pm 2SD$: 6.3 ± 2 mmol/l) as compared to normal subjects (4.2 ± 0.6). The largest difference was obtained at 9°C (patients: 11.4 ± 3.6; normal subjects: 5.2 ± 0.8). Triangles: FP2 patients; circles: normal subjects. Members III.8 and III.18 exhibited an ambiguous pseudohyperkalaemia (not shown). Kalaemia could not be determined in member II.10. Haemolysis rendenred kalaemia uninterpretable in member III.12.

The 16q23-qter region had been explored using microsatellites D16S511, D16S402, D16S3037, D16S520, D16S498, and D16S3074 in the centromer–telomer direction, according to procedures described previously.[5]

As linkage with chromosome 16 was excluded, a gene scan was undertaken. The ABI PRISM Linkage Mapping Set v2 (Perkin-Elmer, Foster City, CA, USA) was used under the conditions suggested by the manufacturer. Additional markers were used in order to refine the region of interest. An aliquot of each PCR reaction was run on an ABI PRISM 3100 DNA sequencer and results were processed by GENESCAN software. Allele assignation was carried out using the Genotyper™ software. Statistical analysis was performed on the basis of an autosomal disease with complete penetrance. Pairwise linkage analysis was performed using the MLINK program version 5.1 from the LINKAGE computer package.[6] Values for maximum LOD score were calculated with the ILINK program from the same computer package. (The approximate 95% confidence limits for the maximum recombination fraction ($Theta_{max}$) at the maximum LOD score (Z_{max}) were calculated by the 1-LOD-down method.)[6] Alleles were down coded without loss of informativity to reduce the computing time. The multipoint parametric analysis was performed by SimWalk2 v.2.82 using Markov chain Monte Carlo (MCMC) and simulated annealing algorithms.[7]

We sequenced three tentatively candidate genes: the *KCNE4* gene, encoding a potassium voltage-gated channel, Isk-related, the *TUBA1* gene, encoding tubulin $\alpha 1$, and a predicted gene encoding an ABC transporter and located in genomic contig NT_005403 (data not shown).

Results

The carriers were haematologically normal (not shown). Pseudohyperkalaemia was assessed in 23 carriers *vs* 11 noncarriers. At $t = 0$ h, kalaemias (mmol/l; $\pm 2\sigma$) were: 4.3 ± 0.5 *vs* 4.1 ± 0.36. The difference between the means was nonsignificant at $P < 0.001$. After 6 h of incubation at 22°C, kalaemias were 11.3 ± 3.46 *vs* 5.2 ± 0.82 (difference significant at $P < 0.001$). After 6 h of incubation at 9°C, they were 6.2 ± 1.88 and 4.2 ± 0.86 (difference significant at $P < 0.001$) (Figure 1).

Isotopic flux measurements of K^+ influx at 37°C[3] in two carriers showed a slightly increased or normal ouabain + bumetanide-resistant K^+ influx at 0.131 and 0.09 mmol/l cells h (normal, 0.050–010), with a slightly increased ouabain-sensitive (Na^+, K^+) pump rate at 2.66 and 2.78 mmol/l cells h (normal$^{1-2}$), consistent with a minimal leak at 37°C and normal haematology. A 'shallow slope' profile, in which the slope of the temperature dependence in the interval 37–20°C was less than that

seen in the normal, was obtained, which was essentially identical to that seen in FP Edinburgh.[8,9]

The microsatellite study in 16q23-qter excluded the presence of a mutant gene in the 16q23-qter region (Figure 2).

On the other hand, a gene scan allowed to map the searched locus to 2q35–36 (Figure 3). Pairwise linkage analysis showed a maximum Lod score of 5.36 with marker D2S126 (Table 1). Negative results were obtained for all the remaining loci investigated in the genome-wide search. Additional markers (D2S301, D2S2395, D2S1338, D2S2250, D2S163, D2S2197, D2S1363) were employed to narrow down the region of interest; pairwise linkage data strengthened the preliminary data. The highest LOD score (8.46) was obtained with marker D2S1338. Multipoint linkage analysis showed a location score of 11.1 very close to marker D2S2250. Recombinants and multipoint linkage analysis define an *FP2* candidate region of approximately 4 cM.

The 2q35–36 region contains 68 sequences (47 pertaining to cloned genes and 21 to unknown gene).

Sequencing of the *KCNE4* gene, the *TUBA1* gene and the predicted ABC transporter gene failed to reveal any change.

Discussion

The haematological abnormality of familial pseudohyperkalaemia is negligible, but the patients present with

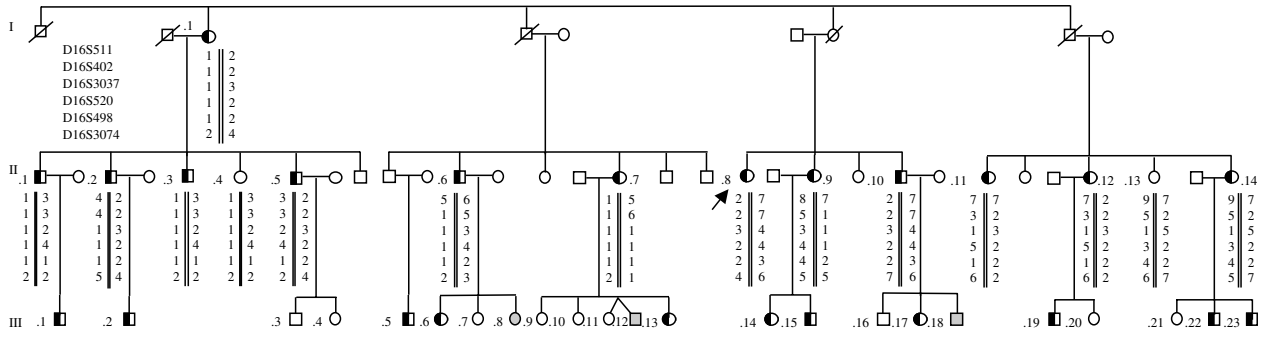

Figure 2 The family tree and the haplotypes (16q23-qter region). White squares and circles: normal members. Black half-squares and half-circles: FP carriers. Arrow: the proband. Several instances allowed to exclude the 16q23-qter region, in particular, the absence of relationship, whichever recombination event might have occurred, between the haplotypes of carriers II.6, II.9 and II.14 and parent carrier I.1.

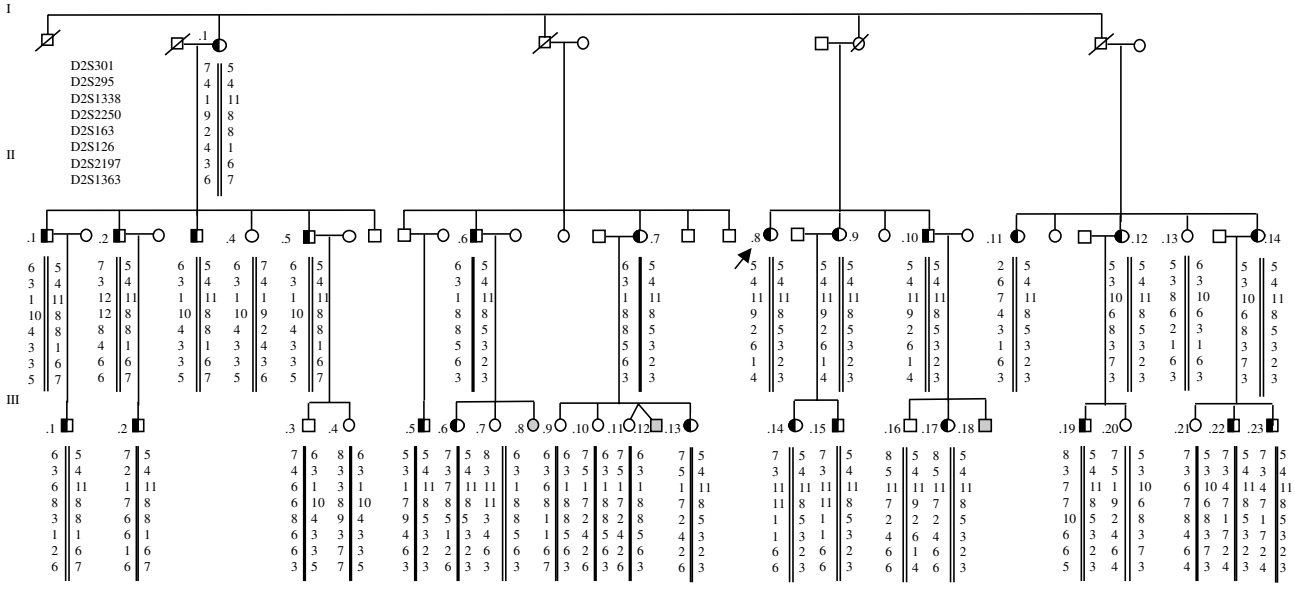

Figure 3 The family tree and the haplotypes (2q35–36 region). White squares and circles: normal members. Black half-squares and half-circles: FP carriers. Arrow: the proband. The 2q35–36 region. Grey circles or squares: nonstudied member (see text). Alleles 11 and 8 of markers D2S1338 and D2S2250, respectively, were constantly associated with FP2.

Table 1 Pairwise LOD scores between FP family and chromosome 2 markers

θ	*0.000*	*0.010*	*0.050*	*0.100*	*0.200*	*0.300*	*0.400*	Z_{max}	θ_{max}
D2S301	−inf	3.93	4.19	3.93	3.02	1.90	0.74	4.20	0.040
D2S295	4.72	4.64	4.28	3.82	2.85	1.82	0.79	4.72	0.001
D2S1338	8.46	8.32	7.73	6.97	5.33	3.51	1.54	8.46	0.001
D2S2250	7.52	7.39	6.85	6.15	4.65	2.99	1.24	7.52	0.001
D2S163	0.68	7.16	7.23	6.71	5.23	3.46	1.47	7.33	0.028
D2S126	−32.29	5.22	5.36	4.93	3.68	2.30	1.10	5.42	0.031
D2S2197	−24.89	4.73	4.86	4.44	3.24	2.01	0.92	4.93	0.031
D2S1363	−inf	1.40	1.97	2.01	1.65	1.10	0.51	2.03	0.082

pseudohyperkalaemia observed in the samples stored for few hours at room temperature. This condition is linked to stomatocytosis as demonstrated in several trees, where stomatocytosis, perinatal oedema and familial pseudohyperkalaemia coexist.[5]

To date, three types of pseudohyperkalaemia devoid of haematological signs have been found, based mostly on the leak–temperature dependence curve: (i) FP Edinburgh[1] and Lille (this work), in which the curve has a shallow slope, (ii) FP Chiswick and Falkirk,[9] in which the curve is shouldered and (iii) FP Cardiff,[10] in which the temperature dependence of the leak shows a 'U-shaped' profile with a minimum at 23°C.

Pseudohyperkalaemia has also been found in association with haematological manifestations. In a class of families,[5] pseudohyperkalaemia was associated with dehydrated hereditary stomatocytosis (DHS). The leak–temperature dependence curve had the same shallow slope as in FP Edinburgh or Lille; only was it slightly translated upward along the *y*-axis. One DHS + pseudohyperkalaemia case mapped to 16q23-qter.[4,11] To which extent this combination maps to 16q23-qter is yet to be assessed.

It is puzzling that an extremely rare trait such as pseudohyperkalaemia can still split into three genetic entities, and that the first of these entities can further split in two subentities. FP Edinburgh stems from *FP1* locus (16q23-qter) and FP Lille from *FP2* locus (2q35–36). These clinical conditions demonstrate the genetic heterogeneity of Familial Psedohyperkalaemia. In consideration of the clinical and laboratory data, we could consider as candidate genes ion channel or protein related to this transport. Tentative sequencing of three plausibly candidate genes in the region of interest yielded negative results.

Acknowledgements

We thank the family for their enthusiastic cooperation and hospitality, Mrs Ingrid Laurendeau, Drs Guy Lalau and Pierre-Oliver Schischmanoff for performing some of the preliminary parts of the investigation, and Pr Gil Tchernia for stimulating discussions. We thank Margaret Chetty for excellent technical assistance. This work was supported by Telethon (prog. E0783, prog. GP0202Y02*), by AIRC, Associazione per la Lotta al Neuroblastoma, Progetto ACRO-CNR and MURST progetti PRIN (Italy) and by MIUR FIRB project (AI), the INSERM (U 473) and the INSERM/AFM (Project no 4MR09F) (JD), and the Sir Jules Thorn Trust for funding (GWS).*

References

1 Stewart GW, Corrall RJM, Fyffe JA, Stockdill GM, Strong JA: Familial pseudohyperkalemia. A new syndrome. *Lancet* 1979; **ii**: 175–177.
2 Iolascon A, Stewart GW, Ajetunmobi JF *et al*: Familial pseudohyperkalemia maps to the same locus as dehydrated hereditary stomatocytosis (hereditary xerocytosis). *Blood* 1999; **93**: 3120–3123.
3 Coles SE, Ho MM, Chetty MC, Nicolaou A, Stewart GW: A variant of hereditary stomatocytosis with marked pseudohyperkalemia. *Br J Haematol* 1999; **104**: 275–283.
4 Vantyghem MC, Dagher G, Doise B *et al*: Pseudo-hyperkaliemie. A propos d'une observation familiale. *Ann Endocrinol* 1991; **52**: 104–108.
5 Grootenboer S, Schischmanoff PO, Laurendeau I *et al*: Pleiotropic syndrome of dehydrated hereditary stomatocytosis, pseudohyperkalemia, and perinatal edema maps to 16q23–q24. *Blood* 2000; **96**: 2599–2605.
6 Ott J: *Analysis of Human Genetic Linkage*. Baltimore: Johns Hopkins University Press, 1991.
7 Mukhopadhyay N, Almsay L, Schroeder M, Mulvihill WP, Weeks DE: Mega2, a data-handling program for facilitating genetic linkage and association analyses. *Am J Hum Genet* 1999; **65**: abs 2474, pp a436.
8 Stewart GW, Ellory JC: A family with mild xerocytosis showing increased cation permeability at low temperatures. *Clin Sci* 1985; **69**: 309–319.
9 Haines P, Crawley C, Chetty M *et al*: Familial pseudohyperkalaemia Chiswick: a novel congenital thermotropic variant of K and Na transport across the human red cell membrane. *Br J Haematol* 2001; **112**: 469–474.
10 Gore DM, Chetty MC, Fisher J, Nicolaou A, Stewart GW: Familial pseudohyperkalaemia Cardiff: a mild version of cryohydrocytosis. *Br J Haematol* 2002; **117**: 212–214.
11 Carella M, Stewart GW, Ajetunmobi JF *et al*: Genomewide search for dehydrated hereditary stomatocytosis (hereditary xerocytosis): mapping of locus to chromosome 16 (q23-qter). *Am J Hum Genet* 1998; **63**: 810–816.