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ARTICLE

Carrier frequency of the V377I (1129G > A) *MVK* mutation, associated with Hyper-IgD and periodic fever syndrome, in the Netherlands

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Hyper-IgD and periodic fever syndrome (HIDS) and mevalonic aciduria (MA) are two autosomal recessive disorders that both are caused by a deficient activity of the enzyme mevalonate kinase (MK) due to mutations in the encoding gene (*MVK*). The most frequently occurring *MVK* mutation, V377I (1129G > A), has been identified exclusively in HIDS patients. Other common mutations have been associated with both HIDS and MA. To estimate the incidence of MK deficiency in the Netherlands, we determined the carrier frequency of the V377I mutation in genomic DNA extracted from anonymised newborn screening cards by PCR-RFLP. We found 14 carriers among 2138 analysed samples (1 : 153). Based on the V377I allele frequency of 42% in patients diagnosed with MK deficiency, the carrier frequency of any *MVK* mutation in the Dutch population can be calculated as 1 : 65. This predicts a disease incidence between 1 in 5196 and 1 in 53 656, which is far more than actually observed. Although under-diagnosis of patients with MK deficiency remains possible, this discrepancy probably is due to a reduced penetrance of V377I homozygosity. Analysis of the distribution of the V377I allele within patients carrying *MVK* mutations revealed that this was not according to the Hardy–Weinberg equilibrium principle, most probably due to an under-representation of V377I homozygotes in HIDS. Homozygotes for V377I might exhibit a much milder phenotype of MK deficiency or no disease-phenotype at all.
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Hyper-IgD and periodic fever syndrome (HIDS, MIM 260920) and mevalonic aciduria (MA, MIM 251170) are two autosomal recessive disorders both caused by a deficient activity of the enzyme mevalonate kinase (MK, E.C. 2.7.1.36).[1–3] Both traits have been classified as auto-inflammatory (or non-infectious inflammatory) disorders.[4–6] MA is a severe and often fatal multi-systemic disease, characterised by psychomotor retardation, failure to thrive, hepatosplenomegaly, anaemia and recurrent febrile episodes.[7] HIDS is a more benign condition, in which patients suffer, as in MA, from recurrent fever episodes associated with lymphadenopathy, arthralgia, gastrointestinal problems and skin rash.[8] Most of the reported HIDS patients to date are of Dutch origin and therefore the disease is known also as Dutch-type periodic fever.[9] However, a main reason for this is most probably the heightened awareness of the disorder in the Netherlands and the inclusion of a specific laboratory test for IgD levels in Dutch patients with periodic fever.

MK enzyme activity in MA is usually below detection levels when measured in cultured skin fibroblasts of MA

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patients.[7,10] In HIDS, however, a residual MK activity varying between 1 and 7% can be measured both in fibroblasts and leukocytes from patients.[3,10–12] MK is an essential enzyme in the isoprenoid/cholesterol biosynthesis pathway and converts mevalonate into 5-phosphomevalonate.[13] This pathway provides cells with isoprenoids that are vital for diverse cellular processes. The main end-products include prenylated proteins, haeme A, dolichol, ubiquinone-10, isopentenyl tRNAs and sterols.[14]

Both MA and HIDS are caused by mutations in the *MVK* gene encoding MK.[2,3,15] In most of the HIDS patients analysed so far, one particular missense mutation, V377I (1129G>A), has been found. Many patients are compound heterozygotes for this common mutation and a second missense mutation that has been identified in both MA and HIDS patients (I268T and H20P).[2,3,11,12,16] The V377I mutation, however, has not been observed in MA patients, strongly suggesting that the V377I mutation is responsible for the HIDS phenotype.

We have developed an efficient PCR–RFLP method for the detection of the V377I mutation and used it to screen a cohort of 2138 random individuals to determine the carrier frequency of the V377I mutation and to calculate the incidence of MK deficiency in the Dutch population. We found a frequency of 1 : 153, which appears higher than predicted from the actual diagnosed cases, probably due to a reduced penetrance of V377I homozygosity.

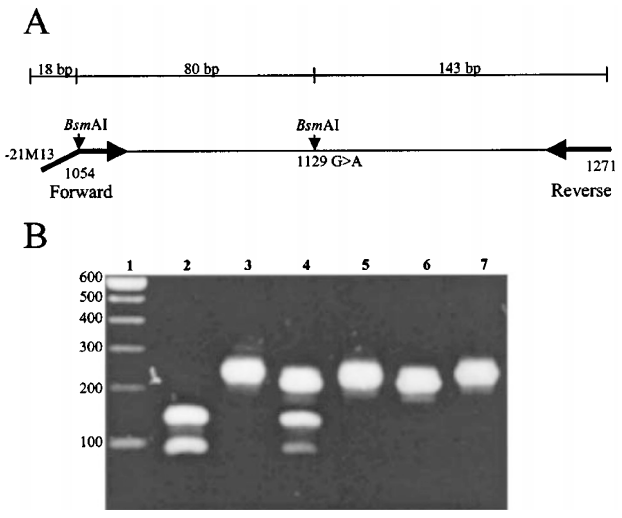

Figure 1 (**a**) Schematic representation of the PCR–RFLP assay for the 1129 G>A mutation. The position of the primers, the restriction sites, the mutation and the length of the PCR-product and restriction fragments are indicated. (**b**) Validation of the assay. Amplification and restriction of a control subject, a patient heterozygous for 1129G>A and a patient homozygous for 1129G>A. Lane 1, DNA molecular weight standard; lane 2, control × *Bsm*AI; lane 3, control uncut; lane 4, 1129G>A heterozygote x *Bsm*AI; lane 5, 1129G>A heterozygote uncut; lane 6, 1129G>A homozygote x *Bsm*AI; lane 7, 1129G>A homozygote uncut.

Materials and methods

DNA isolation and PCR–RFLP assay

DNA was extracted from bloodspots using Chelex (BioRad, Hercules, CA, USA) essentially as described before.[17,18] The isolated DNA was subjected to PCR-restriction fragment length polymorphism (RFLP) analysis to determine the presence of the V377I mutation in the *MVK* gene. This mutation abolishes the recognition sequence for the restriction enzyme *Bsm*AI, thus preventing restriction.[2] For the PCR-RFLP analysis, part of exon 11 containing this mutation was amplified in a 15 μl PCR reaction containing 10 mM Tris/HCl pH 8.4, 50 mM KCL, 1.5 mM $MgCl_2$, 0.01% w/v BSA, 0.2 mM dNTP, 1.5 U Taq polymerase and 0.4 μM of each of the following primers: Forward 5′-tgt aaa acg acg gcc agt gtc tcG AAG TGG AGG CCA CGA AGC AG-3′, Reverse 5′-CCA GCA CAG AGT CGA ACT GCA G-3′. The forward primer introduces a 5′ –21M13 sequence (lower-case letters) and a *Bsm*AI restriction site (underlined). The –21M13 sequence is used for sequencing and characterisation of the PCR product by means of fluorescent labelled terminators according to the manufacturer's protocol (Perkin-Elmer, Foster City, CA, USA). The *Bsm*AI site serves as an internal control for the restriction analysis. A schematic representation of this assay is shown in Figure 1.

The DNA amplification programme started with 2 min of denaturation at 96°C, followed by 5 cycles of 30 s at 96°C, 30 s at 62°C and 30 s at 72°C, and 25 cycles of 30 s at 94°C, 30 s at 62°C and 30 s at 72°C with a final step of 15 min at 72°C. The amplified product was digested overnight at 55°C after the addition of 1.5 μl NEBuffer 3 and 8.5 U *Bsm*AI (New England Biolabs, Beverly, MA, USA). The restriction fragments were analysed on a 2% (w/v) agarose gel by ethidium bromide staining.

Population screening

In the Netherlands, approximately 99% of all newborns (around 200 000 live births yearly) are tested for phenylketonuria, congenital hypothyroidism and adrenogenital syndrome in a nationwide screening programme by means of Guthrie cards. After approval by the Dutch health-care authorities, 2138 anonymised Guthrie cards were obtained from the 14 national screening laboratories, representing the 12 Dutch provinces and the two largest cities (Amsterdam and Rotterdam). The total number of cards selected from each of the 14 screening areas was proportional to the number of live births in each of these regions. In this study, we analysed approximately 1% of the newborns from each of the 14 regions.

Statistical methods

Confidence intervals (CI) were calculated using a method described by Chakraborty *et al*.[19] This method approaches the confidence interval employing a logarithmic trans-

formation in order to make the point estimators almost symmetrically distributed around their expectations.

A χ^2-test was used to determine whether the distribution of *MVK* mutations matched the ratios according to the Hardy-Weinberg equilibrium principle.

Results and discussion

Overview of MVK mutations

As of October 2002, mutational analysis of *MVK* has been performed in 39 unrelated Dutch patients (78 alleles); 34 patients with HIDS and five patients with MA. Combining the results obtained in our laboratory with the data in the international HIDS registry[8] (A Simon, personal communication), we observed that of the 34 Dutch HIDS patients analysed thus far, only four did not carry the V377I allele.[11,12,20,21] Three patients were found to be homozygotes for V377I,[11,12,20,21], two of whom have been confirmed by analysis of parental DNA. Twenty-seven patients were compound heterozygotes for the V377I allele. The second allele in the majority of these patients was one that has been identified also in MA, including the allele H20P (59A>C, seven patients) and the allele I268T (803T>C, nine patients).[11,12,20,21] In addition to these HIDS patients, five Dutch MA patients have been analysed. Four of these were heterozygotes for the A334T (1000G>A) allele, and one patient was homozygous for I268T.[10] From these data the proportion 'R' of the V377I allele among Dutch patients with MK deficiency can be calculated as 42% (33 V377I alleles/78 *MVK* alleles).

The carrier frequency of the V377I MVK mutation and the incidence of MK deficiency in the Netherlands

In order to obtain insight into the actual prevalence of MK deficiency in the Netherlands, we determined the carrier frequency 'Q_{V377I}' of the V377I mutation in the Dutch population. For this purpose we developed an efficient PCR–RFLP assay to screen DNA isolated from newborn Guthrie cards for this mutation. The principle of the method is outlined in Figure 1. The assay was validated on previously characterised DNA samples, which included a control subject, a patient heterozygous for V377I and a patient homozygous for the V377I mutation (Figure 1). Amplification of the fragment yields a product of 241 bp in all samples (lanes 3, 5 and 7). Restriction of a sample with no mutation results in two fragments with the sizes 143 and 80 bp (lane 2). A sample with a homozygous V377I mutation results in a 223 bp fragment (loss of 18 bp –21M13 sequence in front of the internal control *Bsm*AI site, lane 6). A heterozygous sample contains all three fragments (lane 4).

In this study we analysed 2138 anonymised and randomised samples and identified 14 carriers for V377I. No homozygotes were found. Carriers were re-amplified and sequenced in order to confirm the presence of the V377I mutation. The distribution of the carriers over the various geographic regions is shown in Table 1. From these data the carrier frequency of the V377I mutation in the Dutch population 'Q_{V377I}' is estimated to be 1:153 (95% CI of 1:91 to 1:258). From the determined carrier frequency of V377I in the general population and the proportion of this mutation in the patient population, an estimation of the incidence of MK deficiency in the Netherlands is possible via a so-called two-tier mutation survey.[22] To this end, the frequency of any mutant *MVK* allele in the Dutch population 'D' can be calculated by dividing the carrier frequency of V377I in the general population by the proportion of the V377I allele in the patient population (Q_{V377I}/R). This results in a carrier frequency for any disease allele of 1:65 (95% CI of 1:36 to 1:116), which would mean that, based on 200 000 live-births yearly, between four and 38 newborns per year in the Netherlands will have MK deficiency (one per 16 698 newborns with a 95% CI of 5196 to 53 656). These results suggest that MK deficiency would be among the most common recessive disorders in the Netherlands; however, this does not appear to be in line with the actual findings in the Dutch population. Since the first description of HIDS in 1984[23] only 74 Dutch patients have been diagnosed (sibs included). Of these, 47 have been confirmed to result from MK deficiency, which is denoted as classic-type HIDS. Nineteen patients have a variant form of HIDS with normal MK activity.[21] The remaining eight patients are diagnosed with HIDS based on clinical grounds and elevated IgD levels but no MK activity has been analysed. Of the more severe MA phenotype, only five patients are known in The Netherlands.

One conclusion from this may be that many cases of MK deficiency remain undiagnosed. Periodic fever is a widely occurring phenomenon among children and not all cases may be referred to specialist centres. Indeed, in the last

Table 1 The distribution of the V377I allele carriers over the various geographic regions in the Netherlands

Geographic region	*Number of analysed Guthrie cards*	*Observed carriers*	*Carrier frequency*
Limburg	132	0	–
Noord-Brabant	306	1	1:306
Zeeland	88	0	–
Zuid-Holland	366	3	1:122
Noord-Holland	242	0	–
Gelderland	249	2	1:125
Utrecht	151	0	–
Overijssel	144	1	1:144
Drenthe	64	0	–
Groningen	72	1	1:72
Friesland	81	3	1:27
Flevoland	60	2	1:30
Amsterdam	106	1	1:106
Rotterdam	77	0	–
Total	2138	14	1:153 (95% CI 1:91 to 1:258)

few years several new cases have been diagnosed even in adulthood. In addition, not every patient may be diagnosed in our laboratory and/or registered in the international HIDS registry. Furthermore, MA patients may die soon after birth, before diagnosis is made, while it even can not be excluded that there is a higher lethality *in utero*. On the other hand, however, MK deficiency has a diverse and broad clinical spectrum, making reduced penetrance in MK deficiency another possibility. For example, although in many cases MA has a severe disease course, some relatively mild cases have been reported,[7] which are associated with a specific mutation (A334T).[24] Also in HIDS the symptoms may vary, for example the frequency of the fever attacks and the severity of the accompanying symptoms. These differences do not appear to be associated with a specific genotype.[11,20]

The distribution of the V377I allele is not according to the Hardy–Weinberg equilibrium principle

It may be expected that HIDS patients who are homozygous for the V377I allele have more residual MK activity than HIDS patients who are compound heterozygotes for this mutation and a mutation that has been identified in MA (eg H20P or I268T). Therefore, these patients may have a milder phenotype. Our previous identification of a two patient sibship homozygous for the V377I allele, however, indicate that homozygosity for this mild allele can lead to the HIDS phenotype.[12] In addition, two other patients homozygous for V377I have been reported.[11,20]

The evaluation of all Dutch *MVK* mutations yields a value for 'R', the proportion of V377I, of 42%. According to the Hardy–Weinberg equilibrium principle, this gives an 1 : 2.7 : 1.9 expected ratio among MK deficient individuals for patients with two V377I alleles, one V377I allele and no V377I alleles, respectively. A χ^2-test comparing this calculated ratio with the actually observed ratio of 3 : 27 : 9, reveals that it is very unlikely that these distributions are equal ($P<0.01$). Together with the estimated carrier frequency of the V377I allele, which predicts that every year on average between 1 and 6 homozygotes will be born (one per 93 287 newborns with a 95% CI of 32 777 to 265 502), we conclude that V377I is under-represented in HIDS due to an incomplete penetrance. Homozygotes for V377I might exhibit a milder phenotype of MK deficiency or no disease-phenotype at all.

The fact that a significant number of V377I homozygotes may not be diagnosed clinically makes the estimation of MK deficiency in general via the two-tier mutation survey as described above invalid and leads to an overestimation of the true incidence of MK deficiency. Since the mutant alleles of these individuals are not counted when calculating the proportion of V377I among mutant *MVK* alleles ('R') an over-estimation of the carrier frequency for any disease allele will occur. This is probably the main reason for the discrepancy between the observed and estimated incidence of MK deficiency. In this respect, it is also noteworthy that the most prevalent allele in Dutch MA patients, A334T, has never been identified as a compound heterozygous mutation with V377I, whereas the I268T mutation is present homozygous in one MA patient and heterozygous in 10 HIDS patients. This suggests that also the combination of A334T and V377I may have a reduced penetrance. In contrast to other mutations which often affect stability of the MK protein,[3,10,16] A334T encodes a stable MK with a decreased affinity for its substrate mevalonate.[24] This mutant MK protein may be able to stabilise the V377I mutant since MK functions as a dimer.

In conclusion, we determined the carrier frequency of the common V377I *MVK* mutation. Our results show that homozygosity for V377I is underrepresented in HIDS most probably due to incomplete penetrance, making an accurate estimation of the incidence of MK deficiency not possible.

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