Biochemical and genetic aspects of mevalonate kinase and its deficiency
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Citation for published version (APA):
Houten, S. M. (2002). Biochemical and genetic aspects of mevalonate kinase and its deficiency

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Chapter 7

Carrier frequency of the 1129G>A (V377I) MVK mutation, associated with Hyper-IgD Syndrome, in The Netherlands

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Submitted to Molecular Genetics and Metabolism.
Hyper-IgD and periodic fever syndrome (HIDS) and mevalonic aciduria (MA) are two autosomal recessive disorders both 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, 1129G>A (V377I), 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 1129G>A mutation in genomic DNA extracted from anonymized newborn screening cards by PCR-RFLP (frequency 1:153). Based on a frequency of 44% for the 1129G>A allele in patients diagnosed with MK deficiency, the carrier frequency of any MVK mutation can be calculated as 1:68. This predicts a disease incidence between 1 in 5608 and 1 in 58785, which is far more than observed. Although an underdiagnosis of patients with MK deficiency is possible, this great difference probably is due to an incomplete penetrance of 1129G>A homozygosity. Analysis of the distribution of the 1129G>A allele within patients carrying MVK mutations revealed that this was not according to the Hardy-Weinberg equilibrium principle, most probably due to an underrepresentation of 1129G>A homozygotes in HIDS. Therefore, we conclude that 1129G>A has a reduced penetrance. Homozygotes for 1129G>A might have another, yet unknown (milder) phenotype of MK deficiency or no disease-phenotype at all.

Introduction

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 are classified as autoinflammatory (or noninfectious inflammatory) disorders [4-6]. MA is a severe and often fatal multi-systemic disease, characterized by psychomotor retardation, failure to thrive, hepatosplenomegaly, anemia and recurrent febrile episodes [7]. HIDS is a relative 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 are of Dutch origin and therefore the disease is also known as Dutch-type periodic fever [9]. The reason for this is most probably a 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 undetectable when measured in cultured skin fibroblasts of MA 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 [2, 10-12]. MK is an essential enzyme in the isoprenoid biosynthesis pathway and converts mevalonate into 5-phosphomevalonate. This pathway provides cells with isoprenoids that are vital for diverse cellular processes. The main end-products include prenylated proteins, heme A, dolichol, ubiquinone-10, isopentenyl tRNAs and sterols [13].

Both MA and HIDS are caused by mutations in the MVK gene encoding MK [2, 3, 14]. In most of the HIDS patients analyzed so far, one particular missense mutation, 1129G>A (V377I), has been found. Many patients were compound heterozygotes for this common mutation and a second missense mutation that could be identified in both MA and HIDS patients (803T>C and 59A>C) [2, 3, 11, 12, 15]. The 1129G>A has not been described
in MA patients, all together strongly suggesting that the 1129G>A mutation is responsible for the HIDS phenotype.

We used a PCR-RFLP method to screen a cohort of 2138 random individuals to determine the carrier frequency of the 1129G>A mutation and calculate the incidence of MK deficiency in the Dutch population. Furthermore, we discuss the consequences of our findings.

Material and methods

DNA isolation and PCR-RFLP assay

DNA was extracted from bloodspots using Chelex (BioRad) essentially as described before [16, 17]. The isolated DNA was subjected to PCR-RFLP analysis to determine the presence of the 1129G>A mutation (Figure 1) in the MVK gene. This mutation abolishes the recognition sequence for the restriction enzyme BsmAI, 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 MgCl₂, 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'-tg tta aac agc gcc tgc 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), which can be used for sequencing and characterization of the PCR product, and a BsmAI restriction site (underlined), that serves as an internal control for the restriction analysis. A schematic representation of this assay is shown in figure 1.

The DNA amplification program 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 after the addition of 1.5 µl NEBuffer 3 and 8.5 U BsmAI (New England Biolabs) at 55°C. The restriction fragments were analyzed on a 2% (w/v) agarose gel by ethidium bromide staining.

Population screening

In The Netherlands, ~99% of all newborns (about 200,000 live births yearly) are tested for phenylketonuria, congenital hypothyroidism and adrenogenital syndrome in a nationwide screening program by means of Guthrie cards. After approval by the Dutch health-care authorities, 2138 anonymized Guthrie cards were obtained from the 14 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 analyzed ~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. [18]. This method approaches the confidence interval employing a logarithmic transformation in order to make the point estimators almost symmetrically distributed around their expectations.

A χ²-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
Mutational analysis of MVK has been performed in 34 Dutch patients (68 alleles); 30 patients with HIDS and 4 patients with MA. Combining the results obtained in our laboratory and the data in the international HIDS registry [8], we observed that of the 30 Dutch HIDS patients analyzed thus far, only 3 did not have the 1129G>A allele [11, 12, 19]. Three patients were found to be homozygotes for 1129G>A [11, 12, 19], two of whom have been confirmed by analysis of parental DNA. Twenty-four patients were compound heterozygotes for the 1129G>A allele. The second allele in the majority of these patients was one that has been identified also in MA, including the 59A>C allele (H20P, 6 patients) and the 803T>C allele (I268T, 10 patients)[11, 12, 19]. In addition to these HIDS patients, we have analyzed 4 Dutch MA patients. Three of these were heterozygotes for the 1000G>A (A334T) allele, and one patient was homozygous for 803T>C [10]. From these data the proportion “R” of the 1129G>A allele among Dutch patients with MK deficiency can be calculated as 44% (30 1129G>A alleles / 68 MVK alleles).

The carrier frequency of the 1129G>A 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 “P” of the 1129G>A mutation in the Dutch population. For this purpose we developed an efficient PCR-RFLP analysis to screen DNA derived from newborn Guthrie cards for this mutation. The principle of the method is outlined in figure 1. The assay was validated on previously characterized DNA samples, which included a control subject, a patient heterozygous for 1129G>A and a patient homozygous for the 1129G>A mutation (figure 1). Amplification of the fragment yields a product of 241 bp in all these samples (lane 3, 5 and 7). Restriction of a sample with no mutation will result in two fragments with the sizes 143 and 80 bp (lane 2). A sample with a homozygous 1129G>A mutation results in a 223 bp fragment (loss of 18 bp –21M13 sequence in front of the internal control BsmAI site, lane 6). A heterozygous sample will have all three fragments (lane 4).

In this study we analyzed 2138 anonymized and randomized samples and identified a total of 14 carriers for 1129G>A. No homozygotes were found. The estimated carrier frequency of the 1129G>A mutation in the Dutch population “P” is thus estimated to be 1:153 (95% CI of 1:91 to 1:258). From the determined carrier frequency of 1129G>A 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 [20]. To this end, the frequency of any mutant MVK allele in the Dutch population “D” can be calculated by dividing the carrier frequency of 1129G>A in the general population by the proportion of the 1129G>A allele in the patient population (P/R). This results in a carrier frequency for any disease allele of 1:68 (95% CI of 1:38 to 1:122), which would mean that between 3 and 36 newborns per year in The Netherlands will have MK deficiency (1 per 18157 newborns with a 95% CI of 5608 to 58785). These results indicate that MK deficiency would be among the most common recessive disorders, however, this appears not in line with the actual findings in the Dutch population. Since the first description of HIDS in 1984 [21] only 73 Dutch patients have been diagnosed. Of these, 36 have MK deficiency, which is denoted as classic-type HIDS. Thirteen patients have a variant form of HIDS with normal MK activity [22]. The remaining 24 patients are diagnosed with HIDS based on clinical grounds.
and elevated IgD levels. Of the more severe MA phenotype, only 5 patients are known in The Netherlands.

**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 x BsmAI; lane 3, control uncut; lane 4, 1129G>A heterozygote x BsmAI; lane 5, 1129G>A heterozygote uncut; lane 6, 1129G>A homozygote x BsmAI; lane 7, 1129G>A homozygote uncut.

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 centers. Indeed, in the last 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 could be made, while it even can not be excluded that there is a higher lethality in utero. On the other hand, 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 (1000G>A) [23]. 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, 19].

**The distribution of the 1129G>A allele is not according to the Hardy-Weinberg equilibrium principle**

It may be expected that HIDS patients homozygous for the 1129G>A 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 (e.g. 59A>C or 803T>C). Therefore, these patients may have a milder phenotype. Our previous identification of a two patient sibship homozygous for the 1129G>A allele, however, suggested that homozygosity for this mild
The carrier frequency of the 1129G>A mutation in The Netherlands

allele leads to the HIDS phenotype and might have a complete penetrance [12]. In accordance, two other patients homozygous for 1129G>A have been reported [11, 19].

The evaluation of all Dutch MVK mutations yields a value for "R", the proportion of 1129G>A, of 44%. This gives according to the Hardy-Weinberg equilibrium principle an 1 : 2.5 : 1.6 expected ratio among MK deficient individuals for patients with two 1129G>A alleles, one 1129G>A allele and no 1129G>A alleles, respectively. A χ²-test comparing this calculated ratio with the actually observed ratio of 3 : 24 : 7, reveals that it is very unlikely that these distributions are equal (p<0.01). Together with the estimated carrier frequency of the 1129G>A allele, which predicts that every year on average between 1 and 6 homozygotes will be born (1 per 93287 newborns with a 95% CI of 32777 to 265502), we conclude that 1129G>A is underrepresented in HIDS due to an incomplete penetrance. Homozygotes for 1129G>A might have another, yet unknown (milder) phenotype of MK deficiency or no disease-phenotype at all.

The fact that a significant number of 1129G>A 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 incidence of MK deficiency. Since the mutant alleles of these individuals are not counted when calculating the proportion of 1129G>A among mutant MVK alleles ("R") an overestimation 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, 1000G>A, has never been identified as a compound heterozygous mutation with 1129G>A, whereas the 803T>C mutation is present homozygous in one MA patient and heterozygous in 10 HIDS patients. This suggests that the combination of 1000G>A and 1129G>A also may have a reduced penetrance. In contrast to other mutations which often affect stability of the MK protein [3, 10, 15], 1000G>A encodes a stable MK with a decreased affinity for its substrate mevalonate [23]. This mutant MK protein may be able to stabilize the V377I mutant since MK functions as a dimer.

In conclusion, we determined the carrier frequency of the common 1129G>A MVK mutation. Our results show that homozygosity for 1129 G>A is underrepresented in HIDS most probably due to an incomplete penetrance, making an accurate estimation of the incidence of MK deficiency not possible.

Acknowledgements
The authors thank Rebecca Brauner for help with the DNA isolation, Anna Simon for communication on mutations and patients in the International HIDS registry, Joost Frenkel for critical reading the manuscript and Rebecca Holman for help with statistics.

H.R. Waterham is supported by a fellowship of the Royal Netherlands Academy of Arts and Sciences.

References