Diagnostic Value of Urinary Mevalonic Acid Excretion in Patients with a Clinical Suspicion of Mevalonate Kinase Deficiency (MKD)

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Abstract Objective: In patients suffering from mevalonate kinase deficiency (MKD), the reduced enzyme activity leads to an accumulation of mevalonic acid which is excreted in the urine. This study aims to evaluate the diagnostic value of urinary mevalonic acid measurement in patients with a clinical suspicion of mevalonate kinase deficiency.

Methods: In this single-center, retrospective analysis, all patients in whom both measurement of mevalonic acid and genetic testing had been performed in the preceding 17 years have been included. The presence of two pathogenic *MVK* mutations or demonstration of decreased enzyme activity was considered to be the gold standard for the diagnosis of MKD.

Results: Sixty-one patients were included in this study. Thirteen of them harbored two *MVK* mutations; twelve of them showed elevated levels of mevalonic acid. Forty-eight patients did not harbor any *MVK* mutations, yet five of them excreted increased amounts of mevalonic acid. This corresponds to a sensitivity of 92%, a specificity of 90%, a

positive predictive value of 71%, and a negative predictive value of 98%. The positive likelihood ratio is 10 and the negative likelihood ratio is 0.09.

Conclusion: MKD seems very unlikely in patients with a normal mevalonic acid excretion, but it cannot be excluded completely. Further, a positive urinary mevalonic acid excretion still requires *MVK* analysis to confirm the diagnosis of MKD. Therefore, detection of urinary mevalonic acid should not be mandatory before genetic testing. However, as long as genetic testing is not widely available and affordable, measurement of urinary mevalonic acid is a fair way to select patients for *MVK* gene analysis or enzyme assay.

Introduction

Mevalonate kinase deficiency (MKD) is a rare hereditary autoinflammatory syndrome which is inherited in an autosomal recessive manner. MKD has two phenotypes, known as hyperimmunoglobulinemia D and periodic fever syndrome (HIDS) and mevalonic aciduria (MA) (Prieur and Griscelli 1984; van der Meer et al. 1984; Berger et al. 1985). Both phenotypes are characterized by inflammation with fever accompanied by gastrointestinal complaints, lymphadenopathy, arthralgia, myalgia, skin rash, and mucosal ulcers. Besides these inflammatory attacks, patients affected by the more severe phenotype mevalonic aciduria have dysmorphic features, pre- and postnatal growth retardation, and neurological and ocular involvement (Simon et al. 2004).

MKD is caused by mutations in the mevalonate kinase gene *MVK*. Mevalonate kinase is an enzyme that is part of the mevalonate pathway. This pathway produces cholesterol and unsaturated lipid chains, known as isoprenoids

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(van der Burgh et al. 2013). The mevalonate kinase activity is reduced in MKD patients. The enzyme kinase activity varies from 1.8% to 28% in patients with the HIDS phenotype to below 0.5% in patients affected by the MA phenotype (Houten et al. 1999; Cuisset et al. 2001). This enzyme activity does not correlate perfectly with the severity of the disease (Bader-Meunier et al. 2011). The reduced mevalonate kinase activity leads to an accumulation of its substrate, mevalonic acid, which is excreted in the urine. Elevated levels of urinary mevalonic acid are therefore suggestive of MKD. Examination of HIDS patients showed an increased excretion of mevalonic acid during febrile episodes, while normal levels of mevalonic acid were sometimes found between those episodes (van der Burgh et al. 2013; Poll-The et al. 2000).

Thus, urinary mevalonic acid levels are used as a tool in the diagnostic process of MKD. As clinical criteria are lacking, the diagnosis can only be confirmed by demonstration of decreased enzyme activity or by identification of two known pathogenic *MVK* mutations (Ammouri et al. 2007). However, although urinary mevalonic acid excretion is often used in the diagnostic evaluation of suspected MKD patients, the diagnostic value of this urinary analysis has not been investigated yet and remains unclear. This study aims to evaluate the diagnostic value of urinary mevalonic acid excretion.

Methods

This single-center retrospective study included patients analyzed in the Wilhelmina Children's Hospital. We included all patients in whom both measurement of urinary mevalonic acid and *MVK* analysis were performed in the preceding 17 years. A waiver of review was granted by the institutional ethical review board.

Analysis of Urinary Mevalonic Acid

Urinary mevalonic acid samples were analyzed at the department of medical genetics of the University Medical Center Utrecht. We quantified urinary mevalonic acid using isotope dilution technique. For the determination of mevalonic acid, $^2\text{H}_7$ - mevalonolactone as internal standard is used. To convert mevalonic acid to the lactone form, urine was acidified. The acidified sample was extracted twice with ethyl acetate. The organic phase was dried over anhydrous Na_2SO_4 and evaporated under nitrogen at room temperature. The samples are then hydrolyzed with sodium hydroxide. The dry samples were then dissolved in acetone/ethyl acetate dried and derivatized with BSTFA (bis(trimethylsilyl)tri-

fluoroacetamide). Samples are measured using gas chromatography–mass spectrometry (GC–MS) in the positive electron impact mode. The concentration of mevalonic acid in the urine is calculated using a calibration curve and was expressed as mmol/mol creatinine. (Lindenthal and von Bergmann 1994). The excretion of mevalonic acid was compared with age-dependent reference values, validated in our hospital. These reference values were 0.2–1.9 for patients between 0 and 0.5 year, 0.2–1.0 for patients between 0.5 and 1 year, 0.1–0.7 for patients between 1 and 5 years, and 0.1–0.7 for patients >5 years.

Analysis of Mevalonate Kinase Genes

DNA analysis was performed by extracting DNA from whole blood. Primers were designed for the coding exons, including the intron–exon boundaries (available upon request). The fragments were amplified and sequenced on an ABI 3100 automated sequencer (PE Applied Biosystems, Foster City, CA, USA). Data were analyzed with Sequence Pilot (JSI medical systems GmbH, Kippenheim, Germany). The presence of two pathogenic *MVK* mutations or demonstration of decreased enzyme activity was considered to be the gold standard for the diagnosis of MKD.

Data Analysis

Data were analyzed by using Statistical Package for the Social Sciences (SPSS) 20. The specificity, sensitivity, predictive values, and likelihood ratios of excess urinary mevalonic acid for the diagnosis of mevalonate kinase deficiency were calculated. Differences in clinical features between patients with two *MVK* mutations (MKD patients), patients with elevated mevalonic acid but without *MVK* mutations (false positives), and patients with normal mevalonic acid and without *MVK* mutations (true negatives) were analyzed by using Pearson's chi-square test. The ROC analysis was performed with GraphPad Prism 6.

Results

This study included 61 patients with a clinical suspicion of MKD (32 male and 29 female patients, aged 0.4–36 years). Thirteen patients harbored two *MVK* mutations; 12 of them excreted elevated amounts of mevalonic acid. Forty-eight patients harbored no *MVK* mutations; five of them had at least one elevated mevalonic acid excretion (Table 1). The characteristics of all patients are described in Table 2. The distribution of mevalonic acid excretions is displayed in Fig. 1.

Test Results

The characteristics derived from this study led to a sensitivity of 92%, a specificity of 90%, a positive predictive value (PPV) of 71%, and a negative predictive value (NPV) of 98%. The positive likelihood ratio is 10 and the negative likelihood ratio is 0.09. The area under the curve (AUC) of the receiver operating characteristic (ROC) curve is 0.911 (Fig. 2).

Table 1 Overview of results of mevalonic acid excretion

	MK deficient	MK sufficient	Total
Elevated mevalonic acid	12	5	17
Normal mevalonic acid	1	43	44
Total	13	48	61

MK deficient: presence of two *MVK* mutations. MK sufficient: absence of any *MVK* mutation

This resulted in a sensitivity of 92%, a specificity of 90%, a positive predictive value of 71%, and a negative predictive value of 98%.

Positive likelihood ratio 10; negative likelihood ratio 0.09

Clinical Features

Patients with Two MVK Mutations

Thirty-four assessments of mevalonic acid excretion were performed in thirteen MKD patients. In all but one of them, excretion was elevated. The median excretion in patients with *MVK* mutations was 11 mmol/mol creatinine. Enzymatic studies were performed in eight MKD patients, which corresponded to residual activities ranging from 0.12% to 15% (Table 3).

In one patient with a homozygous V377I mutation, mevalonic acid could not be detected in urine collected during a febrile episode. Febrile episodes were accompanied by stomatitis, rash, arthralgia, diarrhea, abdominal pain, and arthritis. Activity of mevalonate kinase in leukocytes was impaired (2–3%). Thus, although mevalonate kinase activity in leukocytes was impaired in this genetically confirmed MKD patient, urinary mevalonic acid level was not increased. At last follow-up, she experienced only mild MKD attacks, without organ damage.

Table 2 Characteristics of patients with MKD, false-positive tests, and true-negative tests

Characteristics	MK-deficient patients		MK sufficient, mevalonic acid↑		MK sufficient, mevalonic acid↓	
	<i>n</i>	(%)	<i>n</i>	(%)	<i>n</i>	(%)
Baseline						
Patients	13		5		43	
Gender						
(M)	5	(38)	3	(60)	24	(56)
(F)	8	(62)	2	(40)	19	(44)
Febrile episodes	13	(100)	5	(100)	40	(93)
Mevalonic acid	Median	Range	Median	Range	Median	Range
Age at test (years)	3.7	0.6–11.8	3.5	1.6–6.7	5.6	0.4–36.6
Median excretion	11	0–5,461	3.3	0.9–8.1	0.1	0.01–0.7
Symptoms	<i>n</i>	(%)	<i>n</i>	(%)	<i>n</i>	(%)
Abdominal pain	13	(100)	4	(80)	20***	(47)
Diarrhea	13	(100)	2**	(40)	12***	(28)
Rash	9	(69)	1	(20)	12**	(28)
Arthralgia	12	(92)	1**	(20)	10***	(23)
Arthritis	6	(46)	0	(0)	4**	(9)
Stomatitis	10	(77)	1*	(20)	8***	(19)
Lymphadenopathy	11	(85)	2	(40)	14***	(33)
Mental retardation	2	(15)	2	(40)	2	(5)
Dysarthria	1	(8)	0	(0)	0	(0)
Retinitis pigmentosa	1	(8)	0	(0)	0	(0)

MK deficient: presence of two *MVK* mutations. MK sufficient: absence of any *MVK* mutation

MK sufficient, mevalonic acid↑ (false positives). MK sufficient, mevalonic acid↓ (true negatives)

Asterisks indicate significant different frequencies compared to MK-deficient patients: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Fig. 1 Distribution of mevalonic acid excretions in patients without *MVK* mutations and with two *MVK* mutations

Fig. 2 The diagnostic accuracy of urinary mevalonic acid displayed in a receiver operating characteristic (ROC) curve. The mevalonic acid excretion is expressed as mmol/mol creatinine. Asterisk represents a cutoff value of 1 mmol/mol creatinine. This value has a sensitivity of 92% and a specificity of 92%. The area under the curve (AUC) is 0.911

Patients with Elevated Urinary Mevalonic Acid, Without *MVK* Mutations

Five patients did not bear any *MVK* mutations, but had at least one elevated measurement of urinary mevalonic acid. Fifteen measurements were performed in these patients. Six measurements were elevated, while nine measurements showed normal excretions of mevalonic acid. All five patients suffered from febrile episodes; in four patients, the episodes started within the first year of life. The enzyme activity was measured in fibroblasts in two patients; in both of them, the activity was normal in comparison to healthy individuals. Further, there were no indications for other metabolic disorders that could explain the elevated mevalonic acid excretion.

Table 3 Characteristics of patients with elevated mevalonic acid excretions

Patient	Mevalonic acid excretion	Age at test	Mutations	Mevalonate kinase enzyme activity
<i>Mevalonate kinase-deficient patients</i>				
1	2.1	3.3	V377I V377I	
2	13.1	0.7	V377I I268T	
3	1.1	4.9	V377I V377I	
4	11.0	1.0	I268T P167L	1%
5	5,461	1.1	A334T A141fs	0.15%
6	5	11.8	V377I R215Q	
7	32	3.7	V377I I268T	
8	27.5	1.6	V377I I268T	2.5%
9	22.8	7.8	V377I H20P	2.5%
10	10	7.9	V377I I268T	2%
11	4	8.8	N205A V321A	15%
12	23.7	0.6	V377I W62X	3.5%
<i>Mevalonate kinase-sufficient patients</i>				
13	1.1	2.7	—	
14	8.1	3.5	—	
15	4.1	6.7	—	Normal
16	0.9	4.4	—	
17	3.3	1.6	—	Normal

Two patients with an elevated measurement suffered from febrile episodes and typical MKD symptoms, such as pharyngitis, arthralgia, lymphadenopathy, diarrhea, abdominal pain, and stomatitis. Two other patients experienced predominantly neurological impairment, accompanied by febrile episodes. These two patients suffered from childhood absence epilepsy and mental retardation, one of them also had ataxia. In one patient with fever and headache without other specific MKD complaints, genetic analysis for Familial Mediterranean fever (FMF), TNF receptor-associated periodic fever syndrome (TRAPS), and MKD was all negative. Currently, she is not suffering from fever anymore, but the headache is still present.

Patients with Normal Urinary Mevalonic Acid and Without *MVK* Mutations

Fifty-four measurements of urinary mevalonic acid were performed in 43 patients. All of them were normal and none of these patients harbored any *MVK* mutations. Among those forty-three, forty patients suffered from febrile episodes. Those patients suffered predominantly from abdominal pain, diarrhea, lymphadenopathy, arthralgia, rash, and stomatitis. However, these were significantly less often seen compared to patients with *MVK* mutations (Table 2).

Discussion

Assessment of mevalonic acid excretion is often performed in patients with clinical features suggestive of MKD. Nonetheless, the diagnostic value of this assessment was unclear, and genetic testing and enzyme assay are still the only manner to definitely diagnose patients with MKD. Therefore, we examined the diagnostic value of this assessment in a group of patients with a phenotype of periodic fever.

In our cohort, patients with an elevated mevalonic acid excretion had a 71% chance to be suffering from mevalonate kinase deficiency. Examination of MKD patients and those with a false-positive test result showed many similarities and some discrepancies as well. Typical MKD symptoms such as abdominal pain, diarrhea, arthralgia, and lymphadenopathy were less prominent in patients with a false-positive test. In two of these patients, MKD was ruled out completely by detecting a normal enzyme activity. Although enzyme activity was not measured in the other three patients with elevated urinary mevalonic acid, the absence of *MVK* mutations made MKD extremely unlikely.

In this study, mevalonic acid could not be detected in one patient with MKD when urine was collected during fever. However, the clinical features of this patient were similar to MKD patients with elevated mevalonic acid excretions. Because there is only one false-negative patient in our series, the negative predictive value is high (98%), but it does show that we cannot rule out MKD in patients with a normal mevalonic acid excretion. We have no explanation for the fact that mevalonic acid was not elevated in this patient.

The reference values used to determine whether the mevalonic acid excretion was elevated are 95% confidence intervals, which means that 5% of all patients excrete higher or lower amounts of mevalonic acid. This might explain why some patients without *MVK* mutations had an elevated mevalonic acid excretion. Other metabolic disorders which might cause an elevated excretion of mevalonic acid, such as cerebrotendinous xanthomatosis and abetalipoproteinemia, were not found in these five patients (Lindenthal et al. 1996; Illingworth et al. 1989).

Currently, mevalonic acid excretion is often collected during febrile episodes (Ammouri et al. 2007). It is assumed that the excretion of mevalonic acid is elevated during febrile MKD episodes. In our study, it was not possible to collect urines exclusively during fever due to the retrospective design of this research. In some cases, it was not possible to trace whether urine samples had been collected during a febrile episode. This might have led to a lower diagnostic accuracy of this test.

Fig. 3 Diagnostic tree in the diagnostic process of MKD

This single-center study was performed in a center with expertise in diagnosis and treatment of MKD. This might have led to a more accurate selection of patients before metabolic and genetic testing are performed and a better handling of the urine samples and thus better test characteristics than would be the case in a hospital with less expertise.

Although this is not a perfect diagnostic study due to the small number of patients and the retrospective design, the rarity of this disease hampers a proper diagnostic study. Therefore, by including all patients suspected of MKD, this study approaches a diagnostic study as close as possible. Based on the reported findings, we made a diagnostic algorithm (Fig. 3).

Several studies have described the phenomenon of elevated mevalonic acid levels in patients with MKD (Simon et al. 2004; Bader-Meunier et al. 2011; Poll-The et al. 2000; Prietsch et al. 2003; Hoffmann et al. 1993; Prasad et al. 2012). Hoffmann et al. have shown in a series of eleven MA patients that highly elevated levels of mevalonic acid occur in urine and that the levels of mevalonic acid in urine correlated with the severity of the disease (Hoffmann et al. 1993). Poll-The et al. described a series of twelve patients with the HIDS phenotype. Mildly increased excretions of mevalonic acid were found in these patients during febrile episodes. In comparison, patients suffering from the MA phenotype excreted higher levels of mevalonic acid (Poll-The et al. 2000). In contrary, Prasad et al. described two cases of severely affected patients with relatively low amounts of mevalonic acid excretion (Prasad et al. 2012).

In conclusion, MKD seems very unlikely in patients with a normal mevalonic acid excretion, but it cannot be excluded completely. Further, a positive urinary mevalonic acid excretion still requires *MVK* analysis to confirm the diagnosis of MKD. Therefore, detection of urinary mevalonic acid

should not be mandatory before genetic testing. However, as long as genetic testing is not widely available and affordable, measurement of urinary mevalonic acid is a fair way to select patients for *MVK* gene analysis or enzyme assay.

One Sentence

Measurement of urinary mevalonic acid is a fair way to select patients for *MVK* gene analysis or enzyme assay, as false-positive or false-negative mevalonic acid measurements can occur.

Compliance with Ethics Guidelines

Conflict of Interest

Joost Frenkel received consultancy fees from NOVARTIS and speaker's fees from SOBI.

Jerold Jeyaratnam, Nienke ter Haar, Monique de Sain-van der Velden, Hans Waterham, and Mariëlle van Gijn declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). A waiver of review was granted by the institutional ethical review board.

Details of the Contributions of Individual Authors

Jerold Jeyaratnam and Nienke ter Haar contributed equally to this study

Jerold Jeyaratnam, Nienke ter Haar, and Joost Frenkel: coordination of the study, data analysis, and draft of the manuscript

Monique de Sain-van der Velden, Hans Waterham, and Mariëlle van Gijn: acquisition of data and final approval of the manuscript

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