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Phenotypic diversity, disease progression, and pathogenicity of *MVK* missense variants in mevalonic aciduria

Heiko Brennenstuhl[1] | Mohammed Nashawi[1,2] | Julian Schröter[3] | Federico Baronio[4] | Lars Beedgen[1] | Florian Gleich[1] | Kathrin Jeltsch[1] | Christina von Landenberg[5] | Silvia Martini[6] | Anna Simon[7] | Christian Thiel[1] | Konstantinos Tsiakas[8] | Thomas Opladen[1] | Stefan Kölker[1] | Georg F. Hoffmann[1] | Dorothea Haas[1] | Unified Registry for Inherited Metabolic Disorders (U-IMD) Consortium and the European Registry for Hereditary Metabolic Disorders (MetabERN)

[1]Division of Neuropaediatrics and Paediatric Metabolic Medicine, Center for Paediatric and Adolescent Medicine, University Hospital Heidelberg, Heidelberg, Germany

[2]Department of Pediatrics, King Abdulaziz University, Jeddah, Saudi Arabia

[3]Division of Pediatric Epileptology, Center for Paediatric and Adolescent Medicine, University Hospital Heidelberg, Heidelberg, Germany

[4]Paediatric Unit, Department of Medical and Surgical Sciences, S. Orsola-Malpighi University Hospital, Bologna, Italy

[5]Department of Neurology, University Hospital Bonn, Bonn, Germany

[6]Neonatal Intensive Care Unit, Department of Medical and Surgical Sciences, S. Orsola-Malpighi University Hospital, Bologna, Italy

[7]Department of Internal Medicine, Radboudumc Expertise Centre for Immunodeficiency and Autoinflammation (REIA), Radboud University Medical Center, Nijmegen, The Netherlands

[8]Department of Pediatrics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Correspondence
Dorothea Haas, Division of Neuropaediatrics and Paediatric Metabolic Medicine, Center for Paediatric and Adolescent Medicine, University Hospital Heidelberg, Im Neuenheimer Feld 430, Heidelberg 69120, Germany.
Email: dorothea.haas@med.uni-heidelberg.de

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Abstract

Mevalonic aciduria (MVA) and hyperimmunoglobulinemia D syndrome (MKD/HIDS) are disorders of cholesterol biosynthesis caused by variants in the *MVK* gene and characterized by increased urinary excretion of mevalonic acid. So far, 30 MVA patients have been reported, suffering from recurrent febrile crises and neurologic impairment. Here, we present an in-depth analysis of the phenotypic spectrum of MVA and provide an in-silico pathogenicity model analysis of *MVK* missense variants. The phenotypic spectrum of 11 MVA patients (age range 0-51 years) registered in the *Unified European Registry for Inherited Metabolic Disorders* database was systematically analyzed using terms of the *Human Phenotype Ontology*. Biochemical, radiological as well as genetic characteristics were investigated. Six of eleven patients have reached adulthood and four have reached adolescence. One of the adolescent patients died at the age of 16 years and one patient died shortly after birth.

Symptoms started within the first year of life, including episodic fever, developmental delay, ataxia, and ocular involvement. We also describe a case with absence of symptoms despite massive excretion of mevalonic acid. Pathogenic variants causing MVA cluster within highly conserved regions, which are involved in mevalonate and ATP binding. The phenotype of adult and adolescent MVA patients is more heterogeneous than previously assumed. Outcome varies from an asymptomatic course to early death. MVK variants cluster in functionally important and highly conserved protein domains and show high concordance regarding their expected pathogenicity.

KEYWORDS

genotypic spectrum, HIDS, mevalonate, mevalonate kinase deficiency, mevalonic aciduria, phenotypic spectrum

SYNOPSIS

The phenotype of adult and adolescent mevalonic aciduria (MVA) patients is more diverse than previously assumed and excretion of mevalonic acid cannot be used to assess the severity of the clinical course of MVA patients.

1 | INTRODUCTION

Mevalonate kinase deficiency (MKD) is an early-onset rare inherited disorder of cholesterol biosynthesis, caused by variants in the *MVK* gene (12q24.11, chr12[hg19] 109,573,255-109,598,125) coding for mevalonate kinase (MVK; E.C. 2.7.1.36).[1] Mevalonate kinase facilitates the phosphorylation of mevalonate to mevalonate-5-phosphate (Figure 1). The main purpose of the mevalonate pathway is the synthesis of the isoprenoids isopentenyl-5-pyrophosphate (IPP) and dimethylallylpyrophosphate (DMAPP), which are substrates for other essential biomolecules such as vitamin D, cholesterol, and steroid hormones, as well as the synthesis of nonsterol isoprenoids.[1,2]

Mevalonic aciduria (MVA, OMIM #610377) and mevalonate kinase deficiency/hyperimmunoglobulinemia D syndrome (MKD/HIDS, OMIM #260920) share the same genetic background of pathogenic *MVK* variants, with most patients having either compound heterozygous or homozygous missense variants. The *MVK* gene is located on the long arm of chromosome 12 (12q24) and spans 11 exons, with a total of 10 coding exons. The transcript length is 2833 base pairs (ENST00000228510.8) encoding a protein of 396 amino acids which was shown to be localized in the cytosol of the cell.[3] MVA patients typically find themselves at the severe end of the phenotypic spectrum.[4,5] The clinical picture of MVA is comprised of early-onset recurrent febrile crises, which are often accompanied by hepatosplenomegaly, lymphadenopathy, arthralgia, and skin rashes. In most cases, neurologic symptoms, such as psychomotor impairment, muscular hypotonia, ataxia associated with cerebellar atrophy, gastrointestinal problems, and ocular symptoms develop later on. Since its first clinical description in the 1980s,[6,7] about 300 patients with MKD have been reported to literature, most of them are found in Western Europe,[8,9] of which approximately 10% are classified as MVA patients.[1] The phenotype of HIDS is milder with recurrent febrile episodes accompanied by lymphadenopathy and arthralgia without neurological impairment.[4,5]

Episodes of metabolic decompensation, as seen in other organic acidurias, are absent in MVA. However, a permanent massive increase of mevalonic acid excretion in urine, independent from febrile crises, is considered pathognomonic.[7] The quantification of mevalonic acid is best done by stable isotope dilution gas chromatographic-mass spectrometric analysis.[10] Additional laboratory findings include high plasma concentrations of immunoglobulin A and D, as well as high urinary excretion of leukotriene E_4.[8] Enzymatic activity of MVK can be measured in fibroblasts and lymphoblasts and MKD/MVA is confirmed by identification of pathogenic variants in the *MVK* gene.

We describe the clinical course of 11 MVA patients and expand the clinical spectrum by reporting two adult patients without any history of periodical symptomatology and another patient without clear MVA associated symptoms, all showing persistent high excretion of mevalonic acid in urine and pathogenic variants in the *MVK* gene. In addition, we describe the distribution of disease-causing missense variants of the *MVK* gene and provide an in-silico pathogenicity model to describe protein regions highly susceptible to damaging variants.

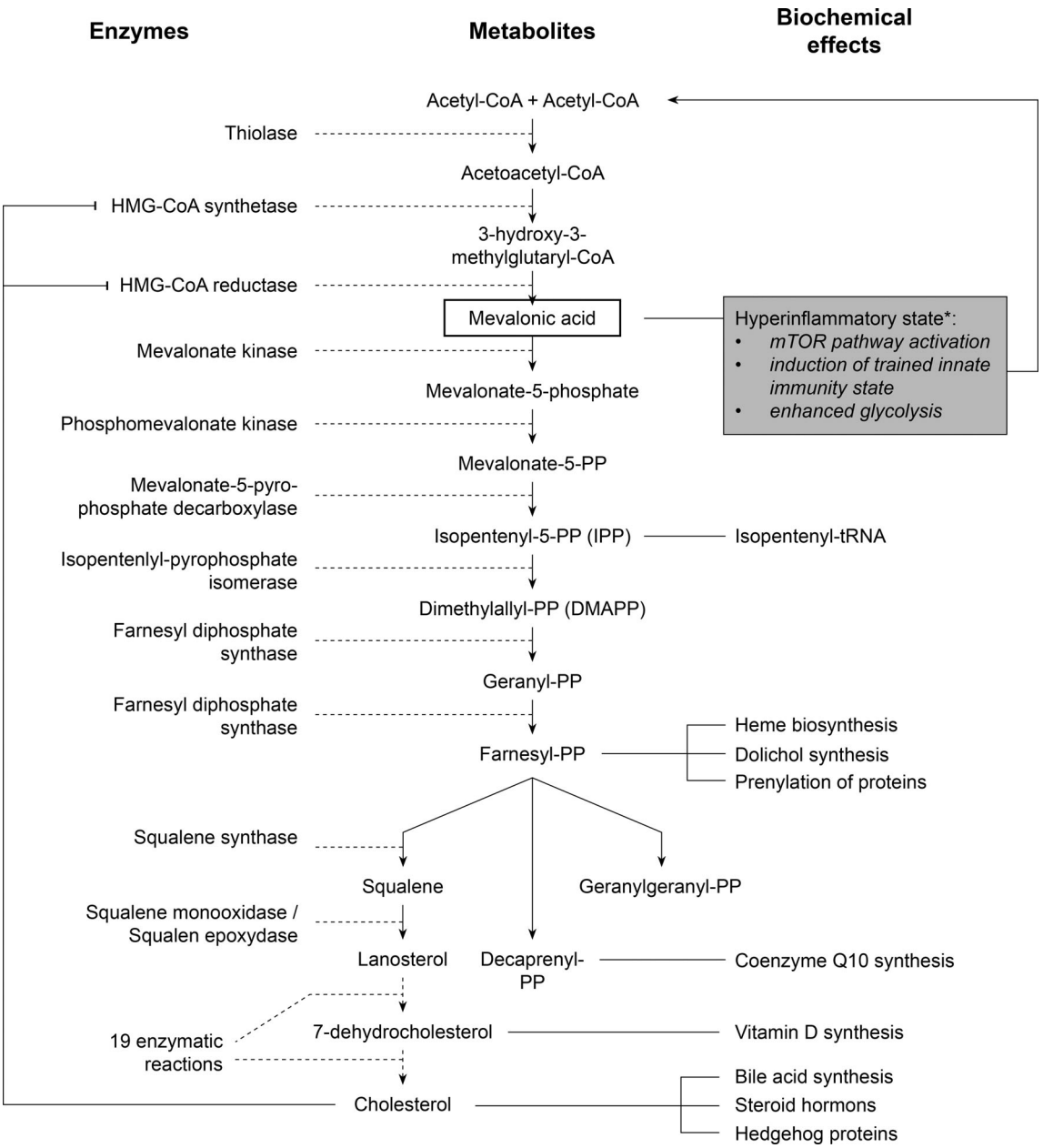

FIGURE 1 Mevalonate pathway and other affected biochemical pathways. Depicted are the enzymes and metabolites of the mevalonate pathway with the corresponding biochemical effects. The biochemical effects of mevalonic acid causing a hyperinflammatory state (marked with asterisk) are based on in vitro data

2 | MATERIALS AND METHODS

2.1 | Unified registry for inherited metabolic disorders

The Unified Registry for Inherited Metabolic Diseases (U-IMD, www.u-imd-registry.org;[11]) is funded by the European Union (EU), via the Consumers, Health, Agriculture and Food Executive Agency (CHAFEA) under the CHAFEA grant HP-PJ-06-2016 (Grant Agreement number: 777259) in the framework of the third Health Programme 2014 to 2020. U-IMD is coordinated by the University Hospital Heidelberg and serves as the official patient registry of the European Reference Network for Hereditary Metabolic Disorders (MetabERN, https://metab.ern-net.eu/). Associated partners are the General University Hospital in Prague, the Bambino Gesù Children's Hospital in Rome, Hospital Sant Joan de Deu in Barcelona and the Udine University Hospital in Udine.

U-IMD implements the European Rare Disease Registry Infrastructure (ERDRI, https://eu-rd-platform.jrc.ec.europa.eu/erdri-description_en), aimed at facilitating a higher level of interoperability between European rare disease registries in general and specifically among registries of European Reference Networks (ERNs). U-IMD gathers comprehensive information on patients with Inborn Metabolic Diseases (IMDs), relying on controlled vocabularies like the nosology of the Inborn Errors of Metabolism Knowledgebase (IEMbase, http://www.iembase.org/), the Human Phenotype Ontology[12] (HPO, release February 27, 2020, https://hpo.jax.org/app/) and the World Health Organization Anatomical Chemical Therapeutic system (WHO ATC, https://www.whocc.no/atc_ddd_index/) among others.

2.2 | Patient characteristics

For semantic interoperability we describe the phenotypic spectrum of MVA patients using the categories and terms of the HPO. We systematically gathered and evaluated clinical, biochemical and radiological data from 11 patients with the genetic and metabolic diagnosis of MVA (see Table 1). Patients originated from Germany, Italy, the Netherlands, and Morocco. Patient 2 has been reported previously as patient 10 in Reference 8; and as patient 2 in Reference 9.[8,9] Patient 3 was previously reported by Hinson et al.[13]

Individual synopses were gathered from clinical reports available within our metabolic center. Laboratory values and radiological data were retrospectively compiled from clinical records.

2.3 | Variant annotation and in-silico modeling of MVK associated pathogenicity scores

To assess the effect of *MVK* missense variants on the MVK protein, 6 high-performance in-silico prediction metascores (VEST4,[14] MetaSVM, and MetaLR,[15] M-CAP,[16] REVEL,[17] and CADD[18]) were annotated using the dbNSFP database v3.0[19] (Table 2). For interpretation, individual threshold values proposed by the authors and the applicable criteria of the ACMG/AMP guideline to assess pathogenicity of individual missense variants were used.[31] To investigate potential differences of the distribution of MVA and MKD/HIDS missense variants, we plotted phenotype-grouped missense variants along a two-dimensional representation of the MVK protein as a lollipop plot. MVA (n = 15) and MKD/HIDS (n = 70) variants were extracted from a recent collection of published records and the ClinVar database.[32,33] To further assess the general susceptibility of the MVK protein to amino acid exchanges, we annotated all biologically possible base exchanges of the *MVK* gene sequence, respectively, the expected amino acid consequence with in-silico pathogenicity scores as previously described.[34,35] Briefly, all biologically possible single base exchanges of the MVK coding sequence (transcript ENST00000228510.8) and their resulting amino acid exchanges were simulated and subsequently annotated with in-silico pathogenicity scores. A pathogenicity heatmap was generated by calculating position-specific mean values of REVEL scores and plotted onto the two-dimensional linearized representation of the MVK protein.

We additionally compared pathogenicity scores of disease-causing *MVK* missense variants vs variants deposited in gnomAD database that were not classified as "pathogenic/Likely pathogenic" or "pathogenic" (n = 51).[36] The results are presented as boxplots with median and 25th and 75th percentiles and whiskers, defined as 1.5 times the interquartile range and individual points for each value. R language version 4.0.3[37] with RStudio IDE version 1.4.1103 (RStudio, Inc.) along with tidyverse/dplyr/ggplot2/ggsingif/ggvenn packages were used for plotting and statistical analysis. Groupwise comparison were done using a Wilcoxon-test, *p*-values are reported as *: $p \leq .05$, **: $p \leq .01$, ***: $p \leq .001$, ****: $p \leq .0001$.

3 | RESULTS

3.1 | Case reports

Detailed clinical summaries of 11 MVA patients with a mean age of 20 years (range 0-51) were compiled. The onset of symptoms was within the first 2 years of life. First symptoms included recurrent febrile episodes, global developmental delay, ataxia combined with cerebellar atrophy, and tapetoretinal degeneration. The most frequent first symptom was episodic fever (73%). Frequency of febrile episodes decreased with age (Table 1). Additionally, patient 7 reported a shift from febrile episodes toward episodes with more generalized malaise. Cerebellar ataxia developed in 7/11 patients, with the first symptoms reported typically after the second year of life. Ocular symptoms, when present, developed early during the disease course. One recently identified patient revealed cerebral cysts, which have not yet been reported to be part of the phenotypic spectrum in MVA (Figure 2). Six patients reached adulthood, four of them currently live in assisted living facilities, two patients live independently. None of the adult patients have children themselves. In the only adult female patient 1, menarche occurred at the age of 12 years with a normal menstrual cycle but reduced menstrual bleeding during months with febrile attacks.

TABLE 1 Clinical and radiological characteristics of 11 MVA patients

	Patient 1[a]		Patient 2[a]		Patient 3		Patient 4		Patient 5		Patient 6		Patient 7		Patient 8		Patient 9		Patient 10[b]		Patient 11[b]	
Gender	Female		Male		Male		Male		Male		Male		Male		Male		Female		Male		Male	
Origin	Germany		Germany		Germany		Germany		Germany		Italy		The Netherlands		Germany		Morocco		Germany		Germany	
Age at symptom onset (in years)	0		2		0		5		1		0		0		0		0		1		0	
Age at diagnosis (in years)	2		2		5		6		1		1		8		1		0		47		48	
Current age (in years)	35		—[c]		26		14		15		16		43		26		—[c]		52		51	
HGVSc	c.1000G>A, c.643C>T		c.1000G>A, c.643C>T		c.1000G>A, c.72dupT		c.1000G>A, c.1000G>A		c.709A>T, c.709A>T		c.60 T>A, c.60 T>A		c.59A>C, c.1000G>A		c.709A>T, c.803 T>C		c.803 T>C, c.803 T>C		c.1000G>A, c.59A>C		c.1000G>A, c.59A>C	
HGVSp	p.(Ala334Thr), p.(Arg215*)		p.(Ala334Thr), p.(Arg215*)		p.(Ala334Thr), p.(Gly25Trpfs*55)		p.(Ala334Thr), p.(Ala334Thr)		p.(Thr237Ser), p.(Thr237Ser)		p.(His20Gln), p.(His20Gln)		p.(His20Pro), p.(Ala334Thr)		p.(Thr237Ser), p.(Ile268Thr)		p.(Ile268Thr), p.(Ile268Thr)		p.(Ala334Thr), p.(His20Pro)		p.(Ala334Thr), p.(His20Pro)	
Visit:	**First**	**Last**	**First**	**Last**	**First**	**Last**	**First**	**Last**	**First**	**Last**	**First**	**Last**	**First**	**Last**	**First**	**Last**	**First**	**Last**	**First**	**Last**	**First**	**Last**
Episodic symptoms:																						
Age at first/last visit (in years)	2	33.9	13.2	16	7.3	23.9	6.1	11.9	6.7	10.8	0.7	13	7	40.9	9.8	26	0.1	—	1	50	0.9	51
Frequency of febrile episodes per year	12	1	12	12	—	—	—	—	3-6	0	3-6	3-6	> 6	≤2[d]	> 6	0	—	—	3-6	≤2	3—6	≤2
Recurrent fever (HP:0001954)	Yes	Yes	Yes	Yes	—	Yes	—	—	Yes	—	Yes	Yes	Yes	—	Yes	—	—	—	Yes	Yes	Yes	Yes
Skin rash (HP:0000988)	Yes	Yes	Yes	Yes	—	Yes	—	—	—	—	—	—	—	—	Yes	—	—	—	—	—	—	—
Lymphadenopathy (HP:0002716)	Yes	Yes	—	—	—	—	—	—	—	—	—	—	—	—	Yes	—	—	—	—	—	—	—
Fluctuating hepatomegaly (HP:0006564)	Yes	Yes	—	Yes	—	—	—	—	—	—	—	—	Yes	—	Yes	—	Yes	Yes	—	—	—	—
Fluctuating splenomegaly (HP:0006268)	Yes	Yes	—	Yes	—	—	—	—	—	—	—	—	Yes	—	Yes	—	Yes	Yes	—	—	—	—
Arthralgia (HP:0002829)	Yes	Yes	—	Yes	—	—	—	—	Yes	Yes	Yes	Yes	—	—	—	—	—	—	—	—	—	—
Diarrhea (HP:0002014)	Yes	Yes	—	Yes	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Yes	Yes	Yes	Yes
Vomiting (HP:0002013)	Yes	Yes	—	Yes	—	—	—	—	—	—	—	Yes	Yes	—	—	—	—	—	Yes	Yes	Yes	Yes
Neurological symptoms:																						
Global developmental delay (HP:0001263)	Yes	Yes	Yes	Yes	Yes	Yes	—	—	—	—	—	Yes	Yes	Yes	Yes	Yes	—	—	Yes	Yes	Yes	Yes
Cerebellar ataxia (HP:0001251)	Yes	Yes	Yes	Yes	Yes	Yes	—	—	—	—	—	—	Yes	Yes	Yes	Yes	—	—	Yes	Yes	Yes	Yes
Muscular hypotonia (HP:0001252)	Yes	Yes	Yes	Yes	Yes	—	—	—	—	—	—	Yes	—	—	—	—	—	—	—	—	—	—
Growth:																						
Failure to thrive (HP:0001508)	—	—	Yes	Yes	Yes	Yes	—	—	Yes	Yes	Yes	Yes	—	—	Yes	Yes	—	—	—	—	—	—
Short stature (HP:0004322)	—	—	—	—	—	—	—	—	Yes	Yes	Yes	Yes	—	—	—	Yes	—	—	—	—	—	—

TABLE 1 (Continued)

Visit:	First	Last	First	Last	First	Last	First	Last	First	Last	First	Last	First	Last	First	Last	First	Last	First	Last	First	Last
Ocular symptoms:																						
Tapetoretinal degeneration (HP:0000547)	—	Yes	—	Yes	—	—	—	—	—	—	—	—	—	Yes	—	Yes	—	—	—	Yes	Yes	Yes
Cataract (HP:0000518)	—	—	—	Yes	—	—	—	—	Yes	Yes	—	—	—	—	Yes	Yes	Yes	Yes	—	—	—	—
Other symptoms:																						
Acute rhabdomyolysis (HP:0008942)	—	—	—	—	—	—	Yes	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Cardiomyopathy (HP:0001638)	—	—	Yes	Yes	—	—	—	—	—	—	—	—	—	—	—	—	Yes	Yes	—	—	—	—
Radiology findings:																						
Cerebellar atrophy (HP:0001272)	—	Yes	Yes	Yes	—	Yes	—	—	—	—	—	—	Yes	Yes	—	—	—	—	—	—	—	—
Agenesis of cerebellar vermis (HP:0002335)	—	Yes	—	Yes	—	—	—	—	—	—	—	—	—	—	—	Yes	—	Yes	—	—	—	—
Cerebral cortical atrophy (HP:0002120)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		Yes	Yes	—	—	—	—
Intracranial cystic lesion (HP:0010576)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Yes	Yes	—	—	—	—
Medical treatment:																						
Coenzyme Q10	Yes		—		Yes		Yes		Yes		—			—			—		—		—	
Tocopherol (Vitamin E)	Yes		—		Yes		Yes		Yes		—			—			—		—		—	
Ascorbic acid (Vitamin C)	Yes		—		Yes		Yes		Yes		—			—			—		—		—	
Vitamin A	Yes		Yes		Yes		Yes		Yes		—			—			—		—		—	
Folic acid	—		—		—		—		—		—			—			—		—		—	
Canakinumab	—		—		—		75 mg 1 x/month		100 mg 1 x/month			150 mg 1 x/month			150 mg 1 x/month		—		—		—	

[a]Siblings.
[b]Siblings.
[c]Deceased.
[d]Episodic symptoms in patient 7 include fatigue, tiredness, and general malaise. Febrile episodes have not been observed since switching therapy to canakinumab.

TABLE 2 MVA causing MVK missense variants and annotated pathogenicity scores

HGVSc	HGVSp	Chromosomal position (GRCh38.p12)	dbSNP	VEST4 score	MetaSVM score	MetaLR score	M-CAP score	REVEL score	CADD phred	Concordance	Source
c.59A>C	p.(His20Pro)	chr12:109574881	rs104895295	0.968	0.9708	0.9431	0.728938	0.894	26.7	6/6 (100%)	This report,[20-22]
c.60 T>A	p.(His20Gln)	chr12:109574882	rs104895335	0.972	0.8959	0.9288	0.644881	0.861	23.5	6/6 (100%)	This report,[23]
c.104 T>C	p.(Leu35Ser)	chr12:109576023	rs104895313	0.948	1.0177	0.9062	0.428416	0.932	28.5	6/6 (100%)	[24]
c.404C>T	p.(Ser135Leu)	chr12:109581427	rs104895297	0.957	0.8502	0.837	0.419958	0.845	24.8	6/6 (100%)	[25]
c.439G>A	p.(Ala147Thr)	chr12:109581462	rs104895363	0.964	1.003	0.9017	0.394554	0.883	25.6	6/6 (100%)	[26]
c.709A>T	p.(Thr237Ser)	chr12:109590802	rs104895366	0.937	0.8671	0.8962	0.459536	0.883	25.6	6/6 (100%)	This report,[27]
c.721C>T	p.(Arg241Cys)	chr12:109590814	rs758427037	0.948	1.0457	0.9241	0.542296	0.941	32	6/6 (100%)	[25]
c.728C>T	p.(Thr243Ile)	chr12:109590821	rs104895314	0.979	1.0712	0.938	0.589753	0.949	24.7	6/6 (100%)	[13,20-22,28]
c.790C>T	p.(Leu264Phe)	chr12:109591262	rs104895315	0.785	0.1505	0.7823	0.189276	0.752	16.66	5/6 (83.3%)	[29]
c.794T>C	p.(Leu265Pro)	chr12:109591266	rs104895316	0.982	1.005	0.9108	0.689978	0.926	24.9	6/6 (100%)	[13,20-22,28]
c.803T>C	p.(Ile268Thr)	chr12:109591275	rs104895304	0.811	1.0605	0.879	0.483788	0.885	23.7	6/6 (100%)	This report,[22]
c.902A>C	p.(Asn301Thr)	chr12:109595044	rs121917789	0.983	1.0791	0.8916	0.549926	0.892	29.2	6/6 (100%)	[30]
c.928G>A	p.(Val310Met)	chr12:109595070	rs104895319	0.796	1.1039	0.9392	0.468391	0.818	25	6/6 (100%)	[20-22]
c.976G>A	p.(Gly326Arg)	chr12:109595118	rs104895308	0.872	1.0457	0.9283	0.535754	0.903	25.2	6/6 (100%)	[29]
c.1000G>A	p.(Ala334Thr)	chr12:109595142	rs104895317	0.892	1.1076	0.9601	0.469035	0.862	27.1	6/6 (100%)	This report,[13,28]

Note: Interpretation as pathogenic variants: VEST 4 score ≥0.5, MetaSVM score ≥0, MetaLR score ≥0.5, M-CAP score ≥0.025, REVEL score ≥0.4, and CADD phred ≥20. Red means the variant is interpreted as pathogenic by the respective score. Green means the variant is interpreted as not clearly pathogenic.

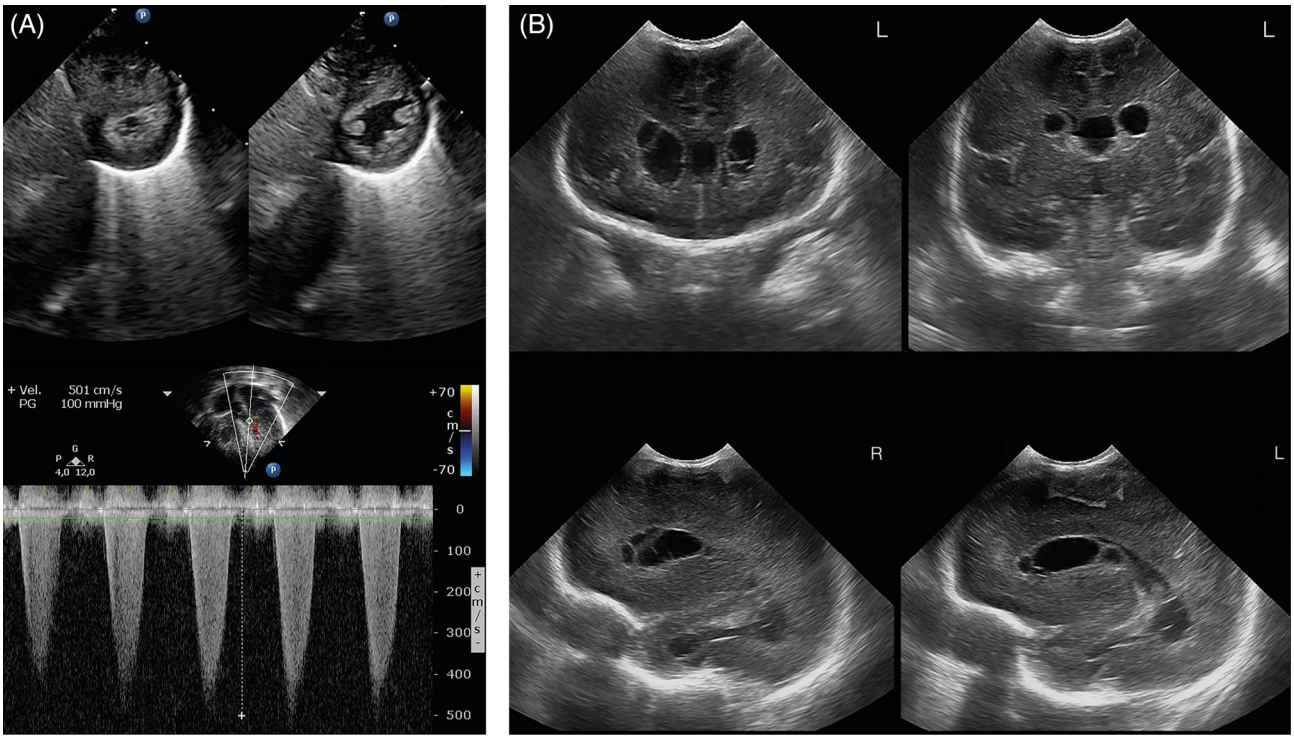

FIGURE 2 Sonographic findings in patient 9. (A) Echocardiography showing hypertrophic interventricular septum with left-ventricular noncompaction pattern (left, systole; right, diastole) and left ventricular output tract pressure gradient (PG). (B) Cerebral ultrasound showing multiple periventricular cysts, hyperechoic periventricular white matter and poor gyration. Top line: coronal views; bottom line: sagittal views. L: left, R: right

3.2 | Severe phenotype with premature death

Patient 2 was diagnosed with MVA at age 1.5 years, shortly after the diagnosis was established in his older sister (patient 1). Both siblings were shown to be compound heterozygous for c.643C>T;p.(Arg215*) and c.1000G>A; p.(Ala334Thr). The clinical course until the age of 14 has been reported previously.[8,9] During the first 1.5 years of life, the patient developed according to his age. Afterward, febrile episodes occurred every 4 weeks and were accompanied by a morbilliform rash, hepatosplenomegaly, lymphadenopathy, vomiting, and diarrhea. An initial MRI of the brain at the age of 2 years was unremarkable. However, at the age of 4.5 years, cranial MRI revealed cerebellar atrophy, followed by a progressive decline of motor abilities and development of ataxia, resulting in a permanent need for a wheelchair. Ophthalmologic investigation revealed nuclear cataracts and signs of retinal dystrophy. Treatment consisted of a combination of vitamins A, C, and E, and CoQ_{10}. The frequency of febrile episodes decreased only temporarily in late childhood and increased again with higher intensity in adolescence. He developed severe kyphoscoliosis and bronchial obstruction, ultimately leading to obstructive lung disease. A muscle biopsy sample revealed type 1 and 2 muscle fiber hypotrophy; however, no functional anomalies of respiratory chain enzymatic function were detected. The patient died at the age of 16 years due to pneumonia with exhaustion of the respiratory musculature and the necessity of artificial ventilation. High concentration of mevalonic acid in urine (average 2687 mmol/mol creatinine, range from 38 to 5060 mmol/mol creatinine [reference 0-0.49 mmol/mol creatinine]) were documented (Table 3) and enzyme activity was measured in fibroblasts with a residual function of 2%.

Patient 9 was the first child of consanguineous parents from Morocco. She had been antenatally diagnosed with severe hypertrophic cardiomyopathy at 21 weeks of gestation and was delivered 8 weeks later by cesarean section due to abnormal cardiotocography. Immediately after birth, she developed severe respiratory distress, hypoxemia and metabolic acidosis. Clinical examination revealed facial dysmorphism including prominent frontal bossing, hypertelorism, a triangular-shaped face with low-set ears, and macroglossia. Echocardiography revealed a left ventricular noncompaction pattern with severely impaired cardiac function with need of inotropic support. Cerebral ultrasound showed intraventricular and periventricular cysts (Figure 2).

TABLE 3 Laboratory test results in mevalonic aciduria (n = 11 patients)

Patient	Mevalonic acid (urine)	Total cholesterol	Aspartate aminotranferase	Immuno-globuline D	Leukotriene E_4	Ubiquinone 50
1	6286	4.2	16	508.2	272	—
2	2687	—	—	1760	263.5	—
3	3190	1.4	—	21.2	—	2125
4	1670	—	—	196.2	—	—
5	366	4.6	10.5	—	—	3853
6	318	2.5	—	12.6	—	2.1
7	2796	—	—	438	—	—
8	605	2.8	52	—	—	—
9	4042	3.4	38.3	—	—	—
10	1748	—	—	—	—	—
11	1403	—	—	—	—	—

Note: Average concentrations are given, mevalonic acid (urine) in mmol/mol creatinine (ref. 0-0.49 mmol/mol creatinine), total cholesterol (ref. age-dependent 2.62-7.67 mmoL/L), Aspartate aminotranferase in U/l (ref. < 37 U/L), immunoglobuline D in mg/l (ref. 1.3-152.7 mg/L), leukotriene E4 in nmol/mol creatinine, ubiquinone-50 in μmol/l (ref. age-dependent <12 months 0.55-1.34 μmoL/L; 1-6 years 0.56-1.67 μmoL/L, >6 years 0.44-1.19 μmoL/L).

Ophthalmologic evaluation revealed bilateral cataract and diffuse retinal hemorrhages. Several days later, she developed an erythematous skin rash. A full metabolic screening revealed excessive excretion of mevalonic acid in urine (4042 mmol/mol creatinine). Due to ongoing inflammation, an anti-inflammatory therapy with methylprednisolone was initiated (2 mg/kg/day) on day 10 but the patient died 11 days later due to cardiac arrest.

3.3 | Moderate phenotype with neurodevelopmental impairment

Patient 7 is the first individual diagnosed with MVA[7] and is now 41 years old. His disease course started with recurrent fever episodes in the first year of life with a frequency of one episode per month. Psychomotor development was delayed with signs of cerebellar ataxia. Ophthalmologic assessment at the age of 7 years revealed tapetoretinal degeneration. The patient was biochemically diagnosed with MVA at the age of 8 years, and is compound heterozygous for the variants c.59A>C;p.(His20Pro) and c.1000G>A;p.(Ala334Thr). Excretion of mevalonic acid remained elevated (on average 2796 mmol/mol creatinine, SD 1003 mmol/mol creatinine), independent from episodes. He also showed increased concentrations of IgD (438 mg/L, reference <153 mg/L). Treatment consisted of a combination of vitamins E and C, and ursodeoxycholic acid. After switching to anti-IL-1 therapy with canakinumab once per month (2 mg/kg) the patient has shown a reduction in frequency and a phenotypic change of episodes to generalized malaise with fatigue, muscle aches, headaches, nausea, dizziness, yet without elevated body temperature.

3.4 | Mild phenotype with absence of characteristic symptoms

Patient 4 showed normal motor and cognitive development. At the age of 6 years he presented with sudden onset of pain and muscular weakness 1 week after he was diagnosed with pyelonephritis for which he had received antibiotic treatment. There was no family history of chronic autoimmune disease or unknown kidney disease. Muscle strength of the lower extremity was decreased with an attenuated patellar tendon reflex. A blood sample revealed massively elevated serum creatine kinase (44 102 U/L; reference <190 U/L), elevated lactate dehydrogenase (1833 U/L; reference <312 U/L), high alanine and aspartate aminotransferases, and slightly elevated CRP upon a normal blood count. While kidney function was normal, high urine excretion of myoglobin 26 604 μg/L (reference <5 μg/L) was noted. Rhabdomyolysis was treated with intravenous fluids. The diagnostic workup revealed influenza B infection. To exclude fatty acid oxidation defects, a metabolic workup was initiated and showed normal acylcarnitines in dried blood but an elevated concentration of mevalonic acid (3257 mmol/mol creatinine) on urinary organic acid analysis. After 7 days of treatment, myopathy improved and laboratory parameters normalized. Rhabdomyolysis was attributed to the viral infection. Upon follow-up, the patient showed

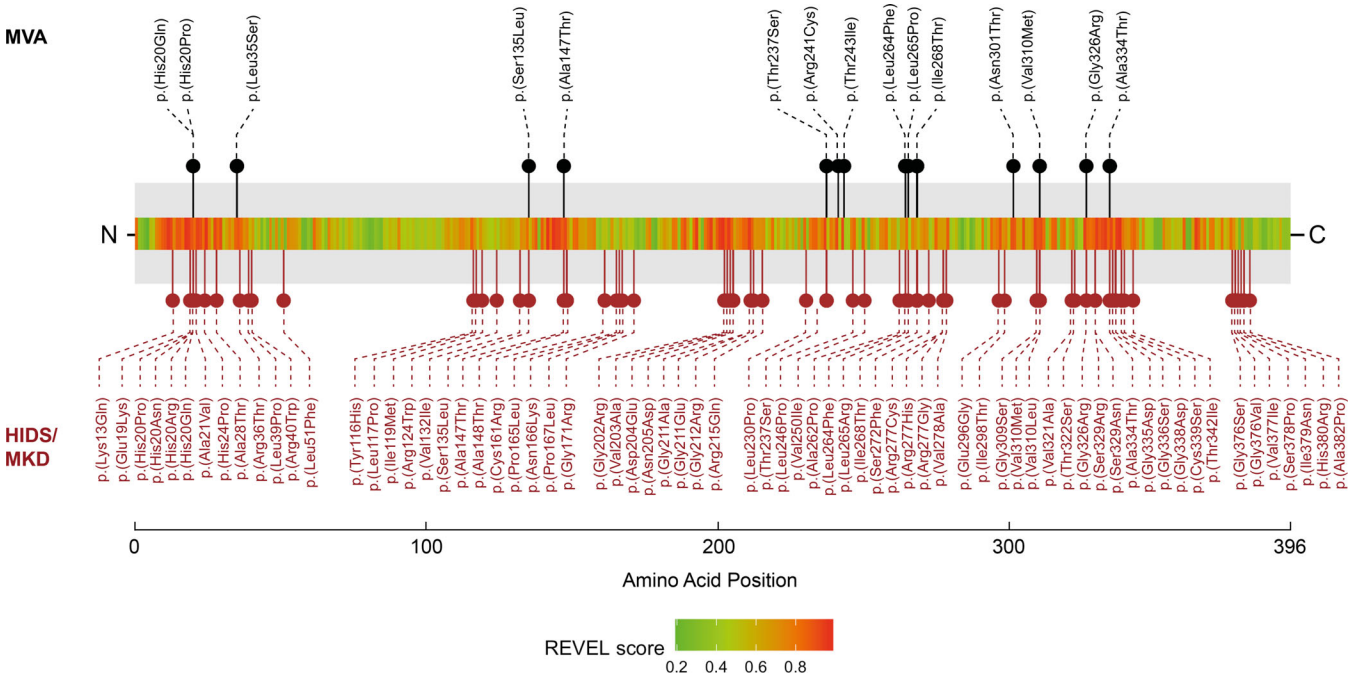

FIGURE 3 Distribution and in-silico pathogenicity score model of disease-causing *MVK* missense variants. A linearized plot of the MVK protein (gray bar) with disease-causing MVA variants (n = 15, above) and MKD/HIDS variants (n = 70, below) is shown. The heatmap shows mean values of the position-specific REVEL scores to visualize areas with low pathogenicity load in green and high pathogenicity load in red

persistently high concentrations of urinary mevalonic acid. Molecular analysis revealed a homozygous variant in the *MVK* gene c.1000G>A;p.Ala334Thr). He was started on a combination of vitamin C, E, and CoQ_{10}. In the following 6 years, he neither experienced any episodic symptoms of MVA nor any other episode of rhabdomyolysis. The laboratory investigations, besides temporarily mildly elevated concentrations of IgD, have been within normal limits; however, the patient shows continuous high excretion of mevalonic acid in urine. Now, at age 13, he attends regular school with an average academic performance.

3.5 | Genotypic spectrum and pathogenicity model of *MVK* variants

The most frequent MVA causing variant in *MVK* in the reported cohort was c.1000G>A;p.(Ala334Thr). We found one individual homozygous for this variant (Patient 4), which is, in contrast to initial reports, not exclusively found in MVA patients, but also in compound heterozygous constellation in MKD/HIDS patients. The second most common variant observed was c.709A>T;p.(Thr237Ser), found in two individuals, again in homozygous and compound heterozygous constellations. Another two patients were reported to have the c.803C>T;p.(Ile268Thr) variant.

Figure 3 shows the distribution of MVA and MKD/HIDS causing variants in *MVK*. A detailed summary of variants and sources can be found in Tables 1 and S1. The majority of MVA-associated variants are found between residues 243 to 334. In addition, two variants were described affecting histidine at position 20, c.59A>T;p.(His20Pro) and c.60T>A;p.(His20Gln), located in close proximity to lysine 13, a residue that was shown to stabilize ATP binding within the catalytic center of the protein.[38] Additional variants were found at position 35, 135, and 147, respectively, the latter also found in individuals with MKD/HIDS phenotype. In Figure 3, a pathogenicity heatmap additionally depicts position-specific mean REVEL scores along the linear protein structure. It is noticeable that the majority of variants are located in regions with high pathogenicity load, associated with high mean REVEL scores. Neither for the MVA nor for the MKD/HIDS phenotype variants are found between amino acid 60 to 110, a region with low scores in the proposed pathogenicity model. A cluster of MKD/HIDS variants including the most common c.1129G>A;p.(Val377Ile) variant is found at the C-terminus, where our model also predicts low pathogenicity.

Overall, the in-silico pathogenicity score analysis of MVA variants shows high concordance of almost 100% for all scores. Only c.790C>T;p.(Leu264Pro) shows a comparatively low CADD score of 16.6. A total of 16 variants are found in the MKD/HIDS group (n = 70) that show at least one in-silico score without clearly predicted pathogenicity. A strikingly low pathogenicity compared to other variants was predicted for variant c.1144G>C;p.(Ala382Pro), for

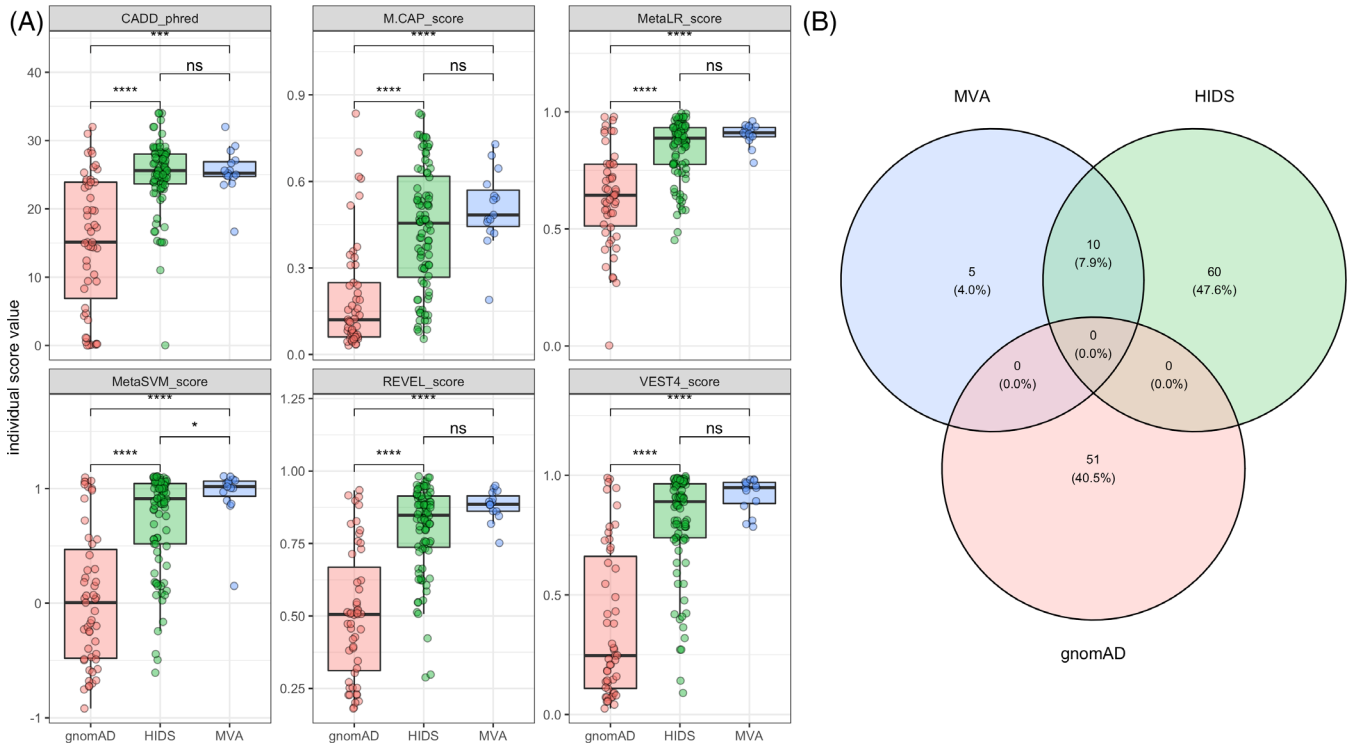

FIGURE 4 Boxplots comparing prediction scores in *MVK* missense variants. (A) Summary statistics shown as boxplots and individual score values as points across *MVK* variants identified in gnomAD healthy controls (n = 51), MKD/HIDS (n = 70), and MVA (n = 15). (B) Venn diagram showing the overlap (n = 10) of MVA (n = 15) and MKD/HIDS (n = 70) causing *MVK* missense variants with no overlap to *MVK* variants annotated in healthy gnomAD controls (n = 51)

which only 1/6 in-silico pathogenicity scores indicated pathogenicity.

Figure 4 shows a visual comparison of scores between *MVK* variants identified in the respective disease groups MVA and MKD/HIDS and variants identified in the general population by gnomAD. A stronger degree of pathogenicity is assigned to the group of MVA variants, while MKD/HIDS variants show a more heterogeneous distribution pattern while being still rated as pathogenic. Variants from gnomAD database, which were used as a group of healthy controls, show the largest overall variability and the lowest pathogenicity scores. This effect was statistically significant for disease-causing variants and control variants for all scores using group comparison with Wilcoxon test (****, $P \leq .0001$ and ***, $P \leq .001$), while group comparison between MVA and HIDS variants was not significantly different.

4 | DISCUSSION

We present a case series of adolescent and adult patients with MVA, extending the phenotypic spectrum of MVA. It is important to increase awareness for this rare disorder as a significant number of patients may remain undiagnosed and not treated efficiently.

In our cohort, symptoms began in early childhood, typically before 2 years of age. The majority of patients (8/11) showed characteristic episodic symptomology with high fever, skin rash, fluctuating hepatosplenomegaly, and lymphadenopathy. In 50% of cases, the episodes decrease in frequency and intensity until adulthood, similar to other periodic fever syndromes. Adult patients can still experience sequels of generalized malaise, headaches, dizziness, and fatigue, with elevated or normal body temperature, as seen in patient 7. Besides episodic symptoms, most patients (7/11) reveal motor and cognitive developmental impairment starting in childhood ultimately leading to cognitive deficits. Motor symptoms include muscular hypotonia (4/11) and cerebellar ataxia (7/11) due to cerebellar atrophy and agenesis of the cerebellar vermis. Myopathy, especially cardiomyopathy, is a major complication with poor prognosis.[8,9,29] The report of a confirmed individual with MVA (Patient 4) without clinical signs of classical MVA or HIDS, despite high and persistent excretion of mevalonic acid in urine and confirmation of pathogenic variants, further extends the phenotypic spectrum of MVA. The episode of rhabdomyolysis leading to the diagnosis in this patient was most likely caused by an influenza B infection as no further episodes occurred within the next 7 years. However, it cannot be excluded that MVA contributed to this symptomatology as myopathy is a well-recognized symptom of MVA.

The wide range of clinical courses cannot be explained by the amount of accumulating mevalonic acid itself but is rather related to the residual enzyme activity. Houten et al. were able to show that MVK activities ranged from 1.8% to 6.9% in patients with MKD/HIDS, but patients with the MVA phenotype had enzyme activities below 0.5%.[20] In fact, the asymptomatic patient described in this report showed an average mevalonic acid excretion of 1670 mmol/mol creatinine, while other severely affected patients excrete less than 1000 mmol/mol creatinine.

Both, MVA and MKD/HIDS, are caused by homozygous or compound heterozygous pathogenic variants of the *MVK* gene. It was previously shown that MKD/HIDS-causing variants of *MVK* cause amino acid changes throughout the entire protein with more than 80% of individuals with MKD/HIDS having the c.1129G>A<;p.(Val377Ile) variant, in most cases in compound heterozygous state.[21,22,29,39,40] Variants causing MVA cluster predominantly around residues 243 to 334 in a highly conserved region of the protein (10/15) which is described to be part of an ATP-binding site.[38] In-silico analysis of pathogenicity scores reveals high score values for several regions of the protein reflecting high mutational susceptibility.

The most frequently observed variant in this report is c.1000G>A;p.(Ala334Thr), predominantly associated with MVA. In the majority of patients this variant was found in a compound heterozygous state, only the least severely affected patient (Patient 4) displayed it in a homozygous state. The overall milder phenotype associated with this variant, could be explained by the observation that neither protein stability nor binding affinity for ATP appear to be affected. Hinson et al. demonstrated that the base exchange from alanine to threonine at position 334 results in a higher K_m for mevalonic acid, which in principle still allows a normal flux of mevalonic acid through the mevalonate pathway,[13] if mevalonate concentration is high enough, explaining the high concentrations of mevalonic acid in our patient. An influence of this region on the stability of mevalonate binding can also be supported by data from crystal structure analyses of the MVK protein.[41,42]

In most patients with the MKD/HIDS phenotype (>80%) the c.1129G>A;p.(Val377Ile) variant has been observed. This variant has been shown to have an impact on folding and stability of the protein.[21,22,27,43,44] Individual reports also mention additional symptoms not typically associated with MKD/HIDS such as global developmental delay, suggesting that these patients fall within the spectrum of MVA rather than MKD/HIDS (patient 14/18 and 21/25 in Reference 29). However, both patients excreted only small amounts of mevalonic acid in urine (6 and 10.3 mmol/mol creatinine during febrile episodes), and a residual enzyme activity of 24% was documented for one of the patients, corresponding clearly to the MKD/HIDS spectrum. It is therefore possible that the reported neurological abnormalities in these patients are not caused by MVK deficiency but have a different origin. Interestingly, only 5/6 (83.3%) of the in-silico pathogenicity scores annotated to this variant describe a damaging effect on the resulting enzyme. These findings underline that shared genomic background does not necessarily imply comparable disease course. Future research efforts should use directed mutagenesis to investigate the influence of single variants of the *MVK* gene in homozygous or compound heterozygous constellations on parameters such as enzyme kinetics or protein stability.

The c.803C>T;p.(Ile268Thr) variant was found in patient 8 in a homozygous state, who presented with severe cardiomyopathy, pulmonary hypertension, cerebellar hypoplasia, cerebral cysts, and hepatosplenomegaly. This patient died at the age of 21 days due to cardiac arrest. The same homozygous variant was reported in one patient who had a severe MVA disease course with premature death at the age of 4.5 months (patient 1[21]). Another patient (patient 103 in Reference 23) with a most likely compound heterozygous constellation with only one reliably detected mono-allelic c.803C>T;p.(Ile268Thr) variant and a residual enzyme activity of 7.3%, classified as MKD/HIDS, supports the hypothesis that this variant likely affects the overall stability of the protein and does not completely abolish function.[21-23] The severe course observed in homozygous variant constellation suggests a higher degree of enzymatic disruption.

Similar variants can lead to substantially different disease courses and outcomes. A possible explanatory approach needs to also consider far-reaching metabolic changes: While the major metabolic product of this pathway is cholesterol, cholesterol concentrations in MVA patients are reported to be within the normal range. Therefore, it is unlikely that dysfunctional cholesterol biosynthesis is the main cause of clinical symptoms. A variety of metabolic dysregulations due to impaired non-sterol isoprenoid synthesis appears more likely to be relevant for the pathophysiology. IPP is needed for isopentenyl-tRNA synthesis for protein translation. Dolichol, a farnesyl derivative, serves as a carrier/membrane anchor for carbohydrate chains in the assembly of glycoproteins. The prenylation of proteins via farnesyl (15 carbon backbone) and geranyl-geranyl (20 carbon backbone) are involved in intracellular signal transduction and regulate growth control and cell cycle. Activation of the IL-1 pathway was shown to be driven by a shortage of geranylgeranylated proteins,[27,44] while impaired prenylation, as seen in HIDS, was described to

lead to RhoA inactivation, thereby causing pyrin inflammasome activation.[45]

Recently, it has been shown that mevalonic acid itself can induce a constitutive trained immunity state of myeloid cells via activation of IGF1-R and mTOR pathways resulting in a hyper-inflammatory state. In T cells, this observation was accompanied by a shift from oxidative phosphorylation to aerobic glycolysis, which further potentiated mevalonic acid production by increased acetyl-CoA synthesis.[46] Despite normal concentrations of CoQ_{10} in MVA patient fibroblasts,[47] it is reasonable to therapeutically generate supra-physiological CoQ_{10} levels in order to reduce intracellular reactive oxygen species content and improve impaired mitochondrial energy metabolism.

Laboratory investigations during febrile crises typically reveal leukocytosis, elevated CRP, and prolonged erythrocyte sedimentation rate. Elevated concentrations of mevalonic acid or its corresponding lactone can be found in plasma and urine and were thought to correlate to a high degree with the severity of the disease course.[1] We report here a patient with high mevalonate excretion in the concurrent absence of symptoms. In future studies, the role of mevalonate excretion as a metabolic marker of disease activity should be further evaluated. High concentrations of circulating IgD and IgA are found in 80% of patients.[48] Genetic confirmation of variants in the *MVK* gene should always be done to confirm the diagnosis.

There is no established treatment regimen for MVA patients. A combination of Coenzyme Q_{10} (CoQ_{10}, 5-10 mg/kg/d), vitamin C (50-60 mg/kg/d), and E (25 mg/kg/d) has been shown to temporarily stabilize the disease course and positively affect psychomotor development in individual cases. The therapeutic use of statins, inhibitors of HMG-CoA reductase, has shown to reduce urinary mevalonate excretion and febrile days in HIDS patients.[4,5] In contrast, a trial of oral statins in two MVA patients had to be discontinued due to severe clinical decompensation.[8] The use of steroids (2 mg prednisone/kg/d) is effective in shortening the duration of crises, similar to what is reported in HIDS patients. Biologicals are commonly used in other periodic fever syndrome conditions. Anakinra and canakinumab, which target the IL-1 pathway, hold potential to control frequency and duration of febrile episodes.[49,50] In HIDS, tocilizumab, a humanized monoclonal antibody against the IL-6 receptor is being successfully used in a growing number of cases leading to clinical and biochemical remission.[51] There are individual reports on a curative approach with allogeneic hematopoietic stem cell transplantation, albeit the treatment is complex, outcomes vary and a risk of relapse remains.[52,53]

The question remains, why some individuals seem to tolerate the enzymatic block and high concentrations of mevalonic acid better than others and further research is needed to unravel the complex pathophysiology of MVA. In this context, Moura and colleagues analyzed the whole exome of 22 patients with known *MVK* variants and 20 patients with recurrent fever in the absence of *MVK* variants, and found differences in the frequency of the rs1450500 single nucleotide polymorphism of the *GRID2* gene (encoding the human ionotropic glutamate receptor delta-2) suggesting a possible role as a phenotype modifier in MVK-associated diseases.[54] Deletions in GRID2 have been reported as a cause for cerebellar ataxia and tonic upgaze, complex spastic paraplegia, and cerebellar atrophy.[55] Hence, it is well worth to further elucidate the role of other genetic modifiers with regards to the phenotype severity in MVA patients.

High concentrations of mevalonic acid in both phenotypes of MKD/HIDS and MVA possibly contribute in large parts to the hyper-inflammatory state. However, the higher disease activity and the presence of neurological symptoms in MVA could be related to a permanent lack of downstream metabolites of the mevalonate pathway. It is likely, that the availability of substrates for glycosylation and prenylation is largely dependent on the residual MVK enzyme activity. To clarify this, additional investigations are required to shed some more light onto these currently not well understood aspects of MVK pathophysiology.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Dorothea Haas, Mohammed Nashawi, and Heiko Brennenstuhl have planned, designed and conducted the study. **Heiko Brennenstuhl drafted the manuscript. Federico Baronio, Christina von Landenberg, Silvia Martini, Anna Simon, Konstantinos Tsiakas, and Georg F. Hoffmann** were involved in the long-term care of patients and collected patient data. **Lars Beedgen, Julian Schröter, and Christian Thiel** were responsible for the genetic characterization of the patients. **Florian Gleich, Kathrin Jeltsch, Thomas Opladen, and Stefan Kölker** are contributors of the U-IMD registry and were responsible for data quality and plausibility check. All authors read and agreed with the manuscript before submission.

ETHICS STATEMENT

The study was approved on July 5, 2018 by the local ethics committee of the coordinating health care provider (ie, University Hospital Heidelberg, application no. S-387/2018) and subsequently adopted by the ethics committees of participating health care providers.

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. Written informed consent was obtained from all patients for being in the study.

ANIMAL RIGHTS

This article does not contain any studies with animal subjects performed by any of the authors.

ORCID

Heiko Brennenstuhl https://orcid.org/0000-0002-6909-0003
Dorothea Haas https://orcid.org/0000-0002-4413-7012

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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