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Basic science

Early-onset gout and rare deficient variants of the lactate dehydrogenase D gene

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Abstract

Objectives: To investigate whether the lactate dehydrogenase D (*LDHD*) gene deficiency causes juvenile-onset gout.

Methods: We used whole-exome sequencing for two families and a targeted gene-sequencing panel for an isolated patient. D-lactate dosages were analysed using ELISA.

Results: We demonstrated linkage of juvenile-onset gout to homozygous carriage of three rare distinct *LDHD* variants in three different ethnicities. In a Melanesian family, the variant was (NM_153486.3: c.206C>T; rs1035398551) and, as compared with non-homozygotes, homozygotes had higher hyperuricaemia ($P = 0.02$), lower fractional clearance of urate ($P = 0.002$), and higher levels of D-lactate in blood ($P = 0.04$) and urine ($P = 0.06$). In a second, Vietnamese, family, very severe juvenile-onset gout was linked to homozygote carriage of an undescribed *LDHD* variant (NM_153486.3: c.1363dupG) leading to a frameshift followed by a stop codon, p.(AlaGly432fsTer58). Finally, a Moroccan man, with early-onset and high D-lactaturia, whose family was unavailable for testing, was homozygous for another rare *LDHD* variant [NM_153486.3: c.752C>T, p.(Thr251Met)].

Conclusion: Rare, damaging *LDHD* variants can cause autosomal recessive early-onset gout, the diagnosis of which can be suspected by measuring high D-lactate levels in the blood and/or urine.

Keywords: juvenile gout, genetics, lactate dehydrogenase D, D-lactate

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Rheumatology key messages
- Rare deficient variants of lactate dehydrogenase D (LDHD) are a cause of autosomal recessive juvenile-onset gout.
- Juvenile-onset gout due to deficient LDHD has now been described in several ethnicities.
- Deficient LDHD was identified in only 1 of 90 tested juvenile gout patients.

Introduction

Elevated blood lactate level has long been known to be associated with less excretion of uric acid and hyperuricaemia [1, 2]. Lactate exists in the human body as two optical isomers: L- and D-lactate. D-lactate is normally nearly absent from human blood and urine because it is metabolized by the enzyme D-lactate dehydrogenase, encoded by the lactate dehydrogenase D (*LDHD*) gene [3]. Autosomal recessive gout caused by a pathogenic variant in the *LDHD* gene encoding deficient D-lactate dehydrogenase resulting in increased D-lactate level was recently identified in a large consanguineous Bedouin-Israeli kindred [4].

Here we report on two families and one isolated case with early-onset gout linked to homozygous carriage of other variants leading to deficient D-lactate dehydrogenase enzyme activity.

Methods

Patients

Studies of the two families were approved by local ethics committees (Comité d'Ethique de Nouvelle Calédonie, and

University of Medicine and Pharmacy at Ho Chi Minh City). Patients were classified as having gout according to the ACR/EULAR classification criteria [5]. Estimated glomerular filtration rate (eGFR) was determined by the Modification of Diet in Renal Disease equation [6].

Sequencing

DNA was extracted from peripheral blood after informed consent was given by all individuals studied. Whole-exome sequencing (WES) was used to identify the genetic cause of familial gout, Sanger sequencing to identify the pathogenic gene variant of extended Melanesian family members and a targeted gene sequencing panel for the isolated case.

WES was performed by the Integragen OncoDNA company (Évry, France). In detail, the exome was captured by using the Human Core Exome Kit (Twist Bioscience, San Francisco, CA, USA). Paired-end sequencing was performed on a NovaSeq analyser (Illumina, San Diego, CA, USA) generating 2*150 pb. Sequencing data were analysed and visualized by using the Sirius interface (Integragen, Évry, France). Sequences were aligned to the human genome reference sequence (UCSC Genome Browser, hg38/GRCh38 build) by using the BWA aligner. Downstream processing involved using the Genome Analysis Tool Kit (GATK), SAMtools and Picard Tools.

For the isolated case genotyping, a targeted gene-sequencing panel for early-onset gout included *LDHD*, *SLC22A12*, *SLC2A9*, *HPRT1*, *ABCG2*, *ALDH16A1* and *UMOD*. The library preparation was based on a hybrid capture system, the surelect QXT kit (Agilent, Les Ulis, France), and the sequencing was performed on a Miseq sequencer (Illumina, San Diego, CA, USA). Sequence results were obtained after aligning fastqs [hg19/GRCh37 (hg37) human genome version], mapping and variant calling by using SeqNext software (JSI Medical Systems, Ettenheim, Germany) based on Smith-Waterman and Burrows Wheeler Aligner algorithms.

Variants were confirmed with Sanger sequencing by using Thermofisher Technologies reagents and software on a Seqstudio sequencer (ThermoFisher, Les Ulis, France).

To assess the frequency and pathogenicity of identified variants, the highest minor allele frequency (MAF) of variants was investigated in the database gnomAD (https://gnomad.broadinstitute.org/); the filtering criteria were maximum MAF <0.05% corresponding to the definition of rare disease in the European Union. Variant pathogenicity was evaluated by using Alamut (SOPHiA Genetics, Lausanne, Switzerland) including variable *in silico* predictive software (SIFT, MutationTaster and Poly-Phen 2) and Combined Annotation Dependent Depletion (CADD) (https://cadd.gs.washington.edu), FATHMM (http://fathmm.biocompute.org.uk) and MetaSVM (Dong). All variants were interpreted and classified according to American College of Medical Genetics (ACMG) guidelines (classification: pathogenic = 5, probably pathogenic = 4, variant of unknown significance = 3).

D-lactate dosage

Dosages of D-lactate in the Melanesian family and the isolated case were analysed in immediately frozen serum and urine samples using ELISA, with a D-lactate colorimetric assay kit (Abcam ab83429).

Statistical methods

Differences between homozygotes and heterozygotes and between homozygotes and non-carriers were tested by Student's *t*-test considering unequal variances. For urine D-lactate, one-sample Student's *t*-test was used because all values for non-homozygotes patients were zero. All reported *P*-values are two-sided; $P < 0.05$ was considered statistically significant. All analyses involved using R 4.1.3 (http://www.R-project.org/).

Results

Family 1

Family 1 was Melanesian, living in the Lifou island of New Caledonia. The family tree is shown in Fig. 1. The two index patients (III-3 and III-4 in Fig. 1) were two sisters in whom gout had developed at ages 13 and 16 years, respectively. When seen at age 25 and 27 years, they both had severe gout with frequent polyarticular flares and ultrasonography-detected double contours at first MTP joints. The one with the earliest age of onset (III-3) also had multiple tophi, gout erosions of a first MTP joint and a boutonnière deformity of her right second PIP joint. WES was performed for the two affected sisters, their parents (who denied consanguinity) and an unaffected brother (III-2 in Fig. 1) and showed that the two affected sisters carried a rare variant in *LDHD* (NM_153486.3: c.206T>C; rs1035398551) at the homozygote level. This variant was at the heterozygote level in both parents and absent in the unaffected brother. It was considered probably damaging according to *in silico* prediction software (SIFT, CADD, Polyphen-2, Fathmm, Meta SVM), and the maximal MAF was very low (Supplementary Table S1, available at *Rheumatology* online). We found no association with any other gene.

The c.206T>C variant in *LDHD* was searched by Sanger sequencing in 13 other extended-family members. One 23-year-old brother of the two early-onset gout sisters (III-5 in Fig. 1) carried the c.206T>C variant at the homozygous level. He described typical monoarticular recurrent gout flares of first MTP joints that started at age 21 years; ultrasonography of MTPs showed double contours, and serum uric acid (SUA) level was high (Table 1), which had been measured at 648 µmol/l at age 3 years during a hospital stay for gastroenteritis. Of note, the brother had had delayed walking and speaking acquisition but subsequently developed normally. Four other heterozygotes were found, of whom two (I-2 and II-6 in Fig. 1) were overweight males with later-onset gout. No variant carrier was found in the eight other genotyped family members, of whom four were overweight males with late-onset gout (Fig. 1 and Table 1).

The three homozygote patients for the c.206T>C variant had very high hyperuricaemia (range: 708–936; $P < 0.02$ as compared with heterozygotes and non-carriers), very low fractional clearance of urate (FCU) (range: 1.13–1.66%; $P < 0.002$), and elevated levels of serum D-lactate (range: 0.3–0.6 mmol/l; $P < 0.02$) and urine D-lactate (range: 7.8–21.5; $P = 0.06$) (Table 1). Heterozygotes or non-carriers had very low levels or no D-lactate in plasma and urine. L-lactate blood and urine levels were in the normal range (Table 1).

Family 2

Family 2 was Vietnamese, living in a remote area of central Vietnam (Fig. 2). The index woman (II-5 in Fig. 2) was seen at
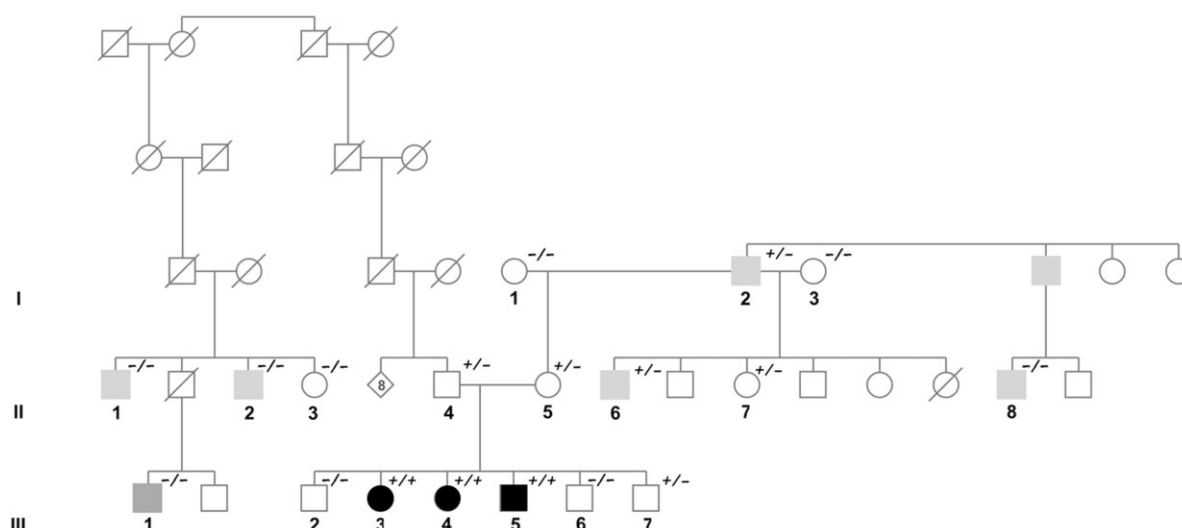
Figure 1. Family tree of the Melanesian family. Black square and circles: juvenile-onset gout; grey squares, late-onset gout: +/+ homozygote carriers of the c.206T>C *LDHD* variant, +/– heterozygote carriers, –/– non-carriers

Table 1. Clinical and biochemical features of the genotyped Melanesian family members, according to their *LDHD* variant status

ID in Fig. 1	Sex	Sampling age (years)	BMI (kg/m^2)	eGFR (ml/min)	Gout/age of onset (years)	SUA max (μmol/l)	FCU	Serum D-lactate[a] (mM/l)	Urine D-lactate[a] (mM/l)	Serum L-lactate[b] (mM/l)
c.206T>C homozygotes										
III-3	F	27	20	108	Yes/16	708	1.13	0.306	21.5	0.090
III-4	F	25	22	98	Yes/13	936	1.66	0.558	7.8	0.058
III-5	M	22	17	85	Yes/18	738	1.14	0.632	18.1	0.089
c.206T>C heterozygotes										
I-2	M	68	31	91	Yes/55	487	2.79	0	0	0.150
II-4	M	50	39	80	No	504	3.43	0.008	0	0.561
II-5	F	47	35	76	No	418	4.27	0.007	0	0.541
II-6	M	42	29	117	Yes/38	433	NA	0	0	1.000
II-7	F	38	31	83	No	292	6.09	0.007	0	0.200
III-7	M	8	16	109	No	254	13	0.009	0	0.615
c.206T>C non-carriers										
I-1	F	65	31	59	No	416	5.80	0.009	0	0.039
I-3	F	62	39	116	No	NA	NA	NA	NA	NA
II-1	M	69	31	69	Yes/65	571	5.38	0	0	1.600
II-2	M	59	31	38	Yes/50	654	3.60	0	0	0.800
II-3	F	55	31	95	No	385	NA	NA	0	ND
II-8	M	35	31	103	Yes/30	477	3.06	0	0	0.200
III-1	M	30	33	88	Yes/23	402	2.19	0	0	0.300
III-2	M	29	24	90	No	371	3.3	0.007	0	0.588
III-6	M	18	32	102	No	445	3.16	0.012	0	0.682

[a] 0 traces.
[b] <1.5 mM/l.
F: female; M: male; eGFR: estimated glomerular filtration rate (Modification of Diet in Renal Disease formula); SUA: serum uric acid level; FCU: fractional clearance of urate; ND: not determined. Normal ranges.

age 33 years at the Vien Gut medical centre (Ho Chi Minh city) with extremely severe, destructive gout that started at age 21 years and had not been treated by urate-lowering drugs. She experienced frequent and severe polyarticular flares and had numerous tophi and destructive arthropathies of the hands and feet, particularly of the two first MTPs (Fig. 3). Ultrasonography of MTPs and knees revealed thick double contour signs. Kidney ultrasonography demonstrated an intensely diffuse hyperechoic medulla. The woman had no history of renal lithiasis. Her BMI was 19.5 kg/m^2; she had no hypertension or diabetes mellitus or dyslipidaemia. SUA level was 545.4 μmol/l, FCU 1.4% and eGFR 69 ml/min/1.73 m^2.

Her family was visited at their home. The youngest brother (II-6 in Fig. 2) had gout that had started at age 9 years and had never received urate-lowering treatment. He had had multiple polyarticular inflammatory flares, and, when we saw him at age 34 years, his joints had been chronically inflamed for a few years; he was wheelchair-bound, with flexion contractures of his elbows and knees. He also had multiple tophi, many of which drained and/or were inflamed (Fig. 4). No radiographs could be taken. Limited biochemical measurements were obtained during our visit: SUA level was 581.5 μmol/l and eGFR 112 ml/min/1.73 m^2. He had gone to school normally, but during the last 3 years exhibited dysarthria, night shakes, memory loss, urine incontinence, inability
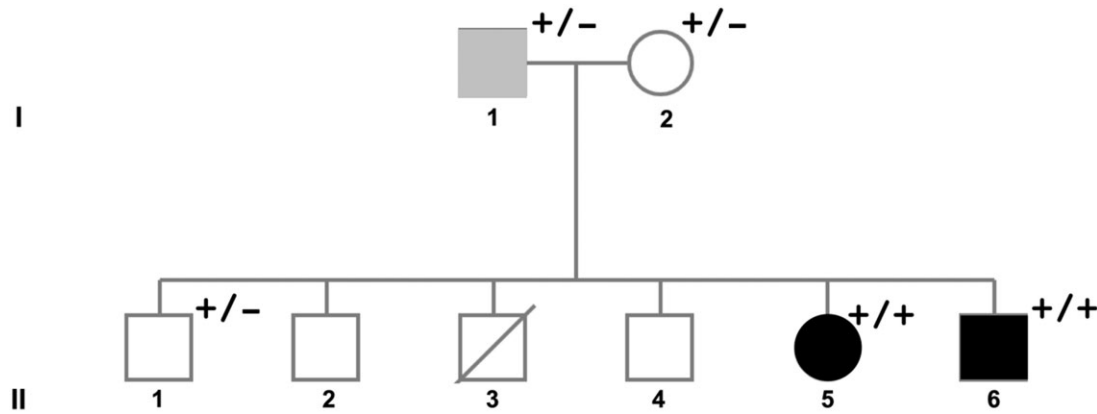
Figure 2. Family tree of the Vietnamese family. Black square and circle: juvenile-onset gout; grey square, late-onset gout: +/+ homozygote carriers of the NM_153486.3: c.1363dupG LDHD variant, +/− heterozygote carriers, −/− non-carriers

Figure 3. Digital photographs (**A**) and radiographs (**B**) of the Vietnamese family index case (II-5 in Fig. 2). Note the massive tophi of the mid-feet and first MTP joint areas and the destructive lytic arthropathy of the first MTPs

to read and count, episodes of confusion, and hypertension. He also had a history of renal colic, with stone expulsion. He received CS chronically and had Cushingoid features. He died a few months after we saw him.

The father had gout that had started at age 73 years, with a small tophus of a second toe, hypertension, coronary heart disease, a history of renal colic, SUA level 526.6 µmol/l and eGFR 37 ml/min/1.73 m². The mother had no gout and normal SUA level (283.9 µmol/l). We also saw the older brother, who, at age 47 years had no gout but was overweight and was found to have asymptomatic hyperuricaemia (414.4 µmol/l).

WES was performed for the two siblings with gout, their father and mother (who denied consanguinity), and the older

brother. An undescribed variant in *LDHD* (NM_153486.3: c.1363dupG) was identified at the homozygous level in the two siblings with juvenile-onset gout and at the heterozygous level in their parents and unaffected brother. This variant led to a frameshift followed by a stop codon, p.(Ala455GlyfsTer58), and was considered pathogenic according to ACMG guidelines. In addition, the two siblings with gout were homozygous for an undescribed frameshift [NM_020407.5: c.1064dupT, p.(Val357GlyfsTer10)] variant in *RHBG* encoding a Rhesus Blood Group family ammonium transporter. Both parents were heterozygous for this variant, which was not identified in the unaffected brother. This gene is not associated with any disease in OMIM or the literature, so it was considered a variant of unknown significance.

Isolated case

The isolated case was a 30-year-old Algerian Caucasian man living in Paris. Gout had started at age 19 years; he had 10–12 polyarticular flares/year and was started on allopurinol (300 mg/day) 2 years after gout onset. SUA level before allopurinol treatment was 720 µmol/l. When we saw him, the BMI was 25 kg/m², and he had no comorbidities, tophus or arthropathy. SUA level was 460 µmol/l and eGFR 130 ml/min/1.73 m². FCU was low, 1.8%. Targeted gene sequencing allowed for identifying a rare variant of *LDHD* [NM_153486.3: c.752C>T, p.(Thr251Met)] at the homozygous level. No other targeted gene variant was found. This homozygous variant was considered damaging according to *in silico* prediction software. The MAF was very low and the variant was associated with the disease. Consequently, the variant was classified as probably pathogenic (Supplementary Table S1, available at *Rheumatology* online). The D-lactate level was <0.5 mmol/l in blood and 6.15 mmol in urine. The man's family was in Algeria and could not be tested.

Discussion

Here we report three distinct rare or undescribed *LDHD* variants encoding for D-lactate dehydrogenase enzyme deficiency linked to autosomal recessive juvenile-onset gout, which developed in lean young patients of both sexes and various ethnicities, with very low FCU. Another *LDHD* variant

Figure 4. Digital photographs of patient III-6 in Fig. 2. Note the helix tophi (**A**, **B**), flexion contractures of chronically inflamed knees (**C**), ulceration and/or inflammation of hand and foot tophi (**D–G**), and swelling of the hands (D, E, G) and left foot (C, F)

[c.1108C>T, p.(Arg370Trp)] was recently reported to cause autosomal recessive gout in a consanguineous Bedouin-Israeli family including young members, but the age of disease onset was not reported [4]. All patients with *LDHD*-linked gout were homozygous for the defective variants, and the urine D-lactate level was high for those who had these measurements. Tested heterozygous individuals did not have elevated D-lactate blood or urine levels and were not affected by early-onset gout, even if later-onset gout developed in some, in the Melanesian family, associated with overweight. This aetiology of juvenile gout is probably rare because we have found *LDHD* variants in only 1 of 90 juveniles with gout so far tested.

In humans, D-lactate results from methylglyoxal metabolism. It can also be produced by colonic bacteria [7] but is normally quasi-absent from the blood because it is metabolized by functional D-lactate dehydrogenase encoded by *LDHD* [3]. The mechanism of juvenile gout in *LDHD*-deficient patients could be explained by elevated blood and urine D-lactate levels resulting from deficient metabolism; this situation would cause increased reabsorption of urate by the proximal tubule urate/anion exchanger URAT1, in exchange for increased elimination of elevated blood D-lactate [8], thus leading to congenital hyperuricaemia. Intriguingly, three patients with other *LDHD* variants carried in the homozygous or compound heterozygous state were reported to have increased D-lactate level with normal or not mentioned uricemia and no gout, in association with various hereditary neurological diseases [3, 9].

A very sick male homozygote from the Vietnamese family did not seem to have obvious neurological problems up to age 28–30 years but was severely affected by neurological features when we saw him at age 34 years. Although we have been unable to further explore his illness because of his inability to go to a well-equipped hospital, he might have been affected by progressive and severe chronic D-lactate acidosis. This disorder, which can be seen in small bowel syndrome, due to fermentation of carbohydrates undigested in the small intestine and delivered to the colon, is indeed known as a source of encephalopathy bouts leading to dysarthria and altered mental functions, similar to our patient's late features [10, 11].

We also found that the two siblings with gout in the Vietnamese family were homozygous for an undescribed variant of the *RHBG* gene [12]. To the best of our knowledge, *RHBG* has so far not been associated with gout.

Our study has limitations. We did not obtain complete biological and imaging data for the Vietnamese family members who lived in a remote area, nor did we demonstrate *in vitro* that the *LDHD* variants we identified affected D-lactate metabolism, in contrast to Drabkin *et al.* who reported this evidence [4]. Nevertheless, we measured serum and urine D-lactate levels in the Lifou family and the Algerian patient: D-lactate levels were greatly and specifically increased in patients carrying homozygous *LDHD* variants. These results advocate strongly for the pathogenic role of *LDHD* variants *in vivo*. We were unable to measure D-lactate levels in the Vietnamese family. However, the frameshift *LDHD* variant that we identified leading to a stop codon is probably located in a catalytic site of the enzyme regarding the previously described frameshift variant in the Monroe *et al.* publication [3], so the hypothesis of a non-functional enzyme is highly probable.

In conclusion, we report three rare or undescribed variants of the *LDHD* gene responsible for autosomal recessive juvenile gout. Following our observation and the report of Drabkin *et al.* [4], we believe that *LDHD* deficiency should

be added to the list of enzymopathies responsible for juvenile gout, which could be suspected by measuring high D-lactates levels in the blood and/or urine.

Supplementary material

Supplementary material is available at *Rheumatology* online.

Data availability

Individual data are available from the first author on request.

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