Hyperuricemia and gout caused by missense mutation in d-lactate dehydrogenase

Max Drabkin,1 Yuval Yoge,1 Lior Zeller,2 Raz Zarivach,2,4 Ran Zalk,4 Daniel Halperin,2 Ohad Wormser,1 Evgenia Gurevich,5 Daniel Landau,6 Rotem Kadir,1 Yonatan Perez,1 and Ohad S. Birk1,7

1The Morris Kahn Laboratory of Human Genetics at the Faculty of Health Sciences, National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer-Sheva, Israel. 2Division of Internal Medicine, Soroka University Medical Center, Beer-Sheva, Israel. 3Department of Life Sciences and 4National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer-Sheva, Israel. 5Rahat Children’s Health Center, Clalit Health Services, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel. 6Department of Pediatrics B and Pediatric Nephrology Unit, Schneider Children’s Medical Center of Israel, Petach Tikva and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel. 7Genetics Institute, Soroka University Medical Center, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

Gout is caused by deposition of monosodium urate crystals in joints when plasma uric acid levels are chronically elevated beyond the saturation threshold, mostly due to renal underexcretion of uric acid. Although molecular pathways of this underexcretion have been elucidated, its etiology remains mostly unknown. We demonstrate that gout can be caused by a mutation in LDHD within the putative catalytic site of the encoded d-lactate dehydrogenase, resulting in augmented blood levels of d-lactate, a stereoisomer of l-lactate, which is normally present in human blood in miniscule amounts. Consequent excessive renal secretion of d-lactate in exchange for uric acid reabsorption culminated in hyperuricemia and gout. We showed that LDHD expression is enriched in tissues with a high metabolic rate and abundant mitochondria and that d-lactate dehydrogenase resides in the mitochondria of cells overexpressing the human LDHD gene. Notably, the p.R370W mutation had no effect on protein localization. In line with the human phenotype, injection of d-lactate into naive mice resulted in hyperuricemia. Thus, hyperuricemia and gout can result from the accumulation of metabolites whose renal excretion is coupled to uric acid reabsorption.

Introduction

Gout is a common rheumatic disease that affects approximately 4% of adults in the United States (1). This inflammatory arthritis most notably affects joints, but also cartilage, bone, and tendons (2, 3). Gout initially presents as inflammatory episodes with increasing frequency, eventually leading to chronic arthropathy (4), and is often associated with renal and cardiovascular comorbidities (5). Gout occurs as a result of deposition of monosodium urate crystals in joints, formed when plasma uric acid levels are chronically elevated (hyperuricemia) beyond the saturation threshold (6). The majority of hyperuricemia cases result from renal underexcretion of uric acid due to various underlying causes, including decreased renal function, side effects of diuretics or other drugs, and impaired function of one of several renal uric acid transporters facilitating its excretion (7–9). Uric acid is the end-product of purine nucleotide metabolism in humans. When it reaches the bloodstream it is typically secreted in exchange for reabsorbed uric acid.

Elevated uric acid levels were evident in both adults (III:5, III:6, III:7, III:9, III:10, III:11) and children (IV:3, IV:4, IV:5). Average plasma uric acid levels were 10.34 ± 1.84 mg/dL and 6.75 ± 0.7 mg/dL in the affected adults and children (Figure 1B), whereas the corresponding normal levels are 3.5–7.2 and 2–6.2 mg/dL, respectively. To assess renal handling of uric acid, we performed urinary analyses on several of the affected individuals. The average urinary uric acid levels measured in 4 affected individuals (III:5, III:7, IV:3, IV:5) were 24.32 ± 15.3 mg/dL (normal levels, 37–92 mg/dL).
Sanger sequencing validated the segregation of the mutation in the family, as expected for autosomal recessive heredity (Figure 2A). The mutation has not been identified in any of the approximately 240,000 genomes in the Genome Aggregation Database (gnomAD) (http://gnomad.broadinstitute.org), or the approximately 117,000 exomes in the Exome Aggregation Consortium (ExAC) database (http://exac.broadinstitute.org), or our in-house exome database of 180 ethnically matched controls. Mutation screening using restriction analysis, which we performed on 92 unaffected members of the particularly highly inbred tribe of the studied kindred, revealed 7 heterozygous carriers of the mutation and no homozygotes (not shown).

The average uric acid levels in a 24-hour urine collection in 2 affected individuals (III:5, III:7) were 125 and 188 ± 68 mg/dL, respectively (normal range, 250–750 mg/dL), whereas creatinine excretion was normal, revealing a substantial reduction in renal clearance of uric acid. In addition, the fractional excretion of uric acid (calculated as the ratio of uric acid clearance to creatinine clearance) in all 5 patients whose samples were assayed (III:5, III:7, III:9, IV:3, IV:5) was 1.4% ± 0.9% (normal value, 7.5%) (8), strongly suggesting that underexcretion of uric acid is the underlying cause of hyperuricemia in this family.

Molecular genetic studies. We performed linkage analysis and homozygosity mapping, testing all 9 affected family members and 6 unaffected controls (II:3, II:4, III:3, III:8, IV:1, IV:2). We identified a single homozygous locus of approximately 12 Mbp on chromosome 16 (between rs150635 and rs2550904) that was shared between all affected family members and none of the healthy ones (maximal logarithm of the odds [LOD] score of 4.8 at rs7193833). We performed whole-exome sequencing on 2 affected individuals who presented with the most severe hyperuricemia (III:7 and III:9, Figure 1A). Following the variant filtration cascade (see Methods), only a single homozygous variant within the genomic locus identified through linkage analysis was found to be shared by both individuals: c.1108C>T in LDHD, encoding d-lactate dehydrogenase. We performed whole-genome sequencing on one of the affected individuals (III:7) to exclude any insertions or deletions in noncoding regions of the homozygous locus identified in the linkage analysis.

Lactic acid exists as 2 enantiomers/stereoisomers — d- and L-lactate — based on the orientation around its asymmetric second carbon atom (15). Normally, lactic acid is found in humans entirely as L-lactate, as mammalian cells almost exclusively produce this form (16). However, d-lactate can be generated in miniscule concentrations via the glyoxalase pathway, which facilitates degradation of methylglyoxal, a byproduct of several fundamental metabolic processes including glycolysis, amino acid degradation, and ketone body catabolism. In this pathway, methylglyoxal is converted to d-lactate following a series of enzymatic reactions. The final step of the pathway is the d-lactate-dehydrogenase-dependent conversion of d-lactate to pyruvate (17), which may then serve as a substrate in a variety of downstream metabolic pathways (18, 19).

The average uric acid levels in a 24-hour urine collection in 2 affected individuals (III:5, III:7) were 125 and 188 ± 68 mg/dL, respectively (normal range, 250–750 mg/dL), whereas creatinine excretion was normal, revealing a substantial reduction in renal clearance of uric acid. In addition, the fractional excretion of uric acid (calculated as the ratio of uric acid clearance to creatinine clearance) in all 5 patients whose samples were assayed (III:5, III:7, III:9, IV:3, IV:5) was 1.4% ± 0.9% (normal value, 7.5%) (8), strongly suggesting that underexcretion of uric acid is the underlying cause of hyperuricemia in this family.

Molecular genetic studies. We performed linkage analysis and homozygosity mapping, testing all 9 affected family members and 6 unaffected controls (II:3, II:4, III:3, III:8, IV:1, IV:2). We identified a single homozygous locus of approximately 12 Mbp on chromosome 16 (between rs150635 and rs2550904) that was shared between all affected family members and none of the healthy ones (maximal logarithm of the odds [LOD] score of 4.8 at rs7193833). We performed whole-exome sequencing on 2 affected individuals who presented with the most severe hyperuricemia (III:7 and III:9, Figure 1A). Following the variant filtration cascade (see Methods), only a single homozygous variant within the genomic locus identified through linkage analysis was found to be shared by both individuals: c.1108C>T in LDHD, encoding d-lactate dehydrogenase. We performed whole-genome sequencing on one of the affected individuals (III:7) to exclude any insertions or deletions in noncoding regions of the homozygous locus identified in the linkage analysis.

Sanger sequencing validated the segregation of the mutation in the family, as expected for autosomal recessive heredity (Figure 2A). The mutation has not been identified in any of the approximately 240,000 genomes in the Genome Aggregation Database (gnomAD) (http://gnomad.broadinstitute.org), or the approximately 117,000 exomes in the Exome Aggregation Consortium (ExAC) database (http://exac.broadinstitute.org), or our in-house exome database of 180 ethnically matched controls. Mutation screening using restriction analysis, which we performed on 92 unaffected members of the particularly highly inbred tribe of the studied kindred, revealed 7 heterozygous carriers of the mutation and no homozygotes (not shown).

Lactic acid exists as 2 enantiomers/stereoisomers — d- and L-lactate — based on the orientation around its asymmetric second carbon atom (15). Normally, lactic acid is found in humans entirely as L-lactate, as mammalian cells almost exclusively produce this form (16). However, d-lactate can be generated in miniscule concentrations via the glyoxalase pathway, which facilitates degradation of methylglyoxal, a byproduct of several fundamental metabolic processes including glycolysis, amino acid degradation, and ketone body catabolism. In this pathway, methylglyoxal is converted to d-lactate following a series of enzymatic reactions. The final step of the pathway is the d-lactate-dehydrogenase-dependent conversion of d-lactate to pyruvate (17), which may then serve as a substrate in a variety of downstream metabolic pathways (18, 19).
acid residues located in proximity to the residues comprising the putative catalytic pocket of the protein and to the arginine residue altered by the novel p.R370W mutation.

Protein localization analyses. It was previously shown that d-lactate dehydrogenase localizes to mitochondria in mammalian cells (18). To verify that in humans the protein is indeed mitochondrial and to determine whether the mutation affected the subcellular localization of the protein, we transfected HEK293 cells with WT and p.R370W-mutant LDHD constructs containing a C-terminal FLAG epitope. We then isolated the mitochondria and cytosolic fractions of the transfected cells and subsequently performed Western blot analysis. Both the WT and p.R370W-mutant LDHD proteins containing the FLAG epitope were detected in the same fraction as VDAC1, which was used as a specific mitochondrial marker, and were not colocalized with the cytosolic marker α-tubulin (Figure 3A). We then confirmed these results using separate constructs in which WT and p.R370W-mutant LDHD were conjugated to red
In urine samples, l-lactate was undetectable in both groups. We found that d-lactate levels in all affected individuals were markedly elevated in both plasma and urine. The average concentration of plasma d-lactate in affected individuals was 3.16 ± 0.63 mM and was undetectable in the healthy controls, whereas the corresponding normal levels are less than 0.043 mM (Figure 4A). In the urine samples, the average levels of d-lactate were 32.08 ± 5.77 mM in the affected individuals versus 0.65 ± 0.65 mM in the healthy controls, whereas the corresponding normal levels are 0.0–0.25 mM (Figure 4A). Thus, although we observed no evident differences in l-lactate levels between affected and healthy individuals, we detected markedly elevated levels of both plasma and urine levels of d-lactate in all affected individuals.

Excess d-lactate causes hyperuricemia in mice. To test whether hyperuricemia may be caused by elevated plasma d-lactate levels, as seen in our patients, we measured plasma uric acid levels in mice following injections of d-lactate. Naive C57BL/6 mice were injected with 200 μL PBS or 3 M d-lactate, and blood samples were taken before and at several time points after injection to monitor plasma uric acid levels. In mice injected with PBS, we observed only slight fluctuations in plasma uric acid levels over time; however, we detected a steady increase in plasma uric acid levels in mice injected with d-lactate, peaking approximately 180 minutes after injection, with an average increase in plasma uric acid levels of 54% ± 0.34% (Figure 4B).

Mass spectrometry. d-lactate dehydrogenase is highly expressed in tissues with increased metabolic activity, and methylglyoxal, which is processed and converted to d-lactate, is a ubiquitous substance that is also abundant in these high-metabolic-rate tissues; thus, d-lactate is constantly produced in humans (22). To our knowledge, there are currently no data describing a particular function of d-lactate in humans. The only known fate of this compound is conversion to pyruvate by d-lactate dehydrogenase or excretion (23, 24). To determine whether this metabolic activity is impaired by the p.R370W mutation as predicted by our structural model, we measured lactate concentrations in plasma and urine samples obtained from 5 affected (III:5, III:7, III:9, IV:3, IV:5) and 4 healthy (II:4, III:1, III:3, III:8) family members. We used a chiral column to differentiate between d-lactate and l-lactate, and a calibration curve was created to determine the absolute concentration of plasma and urine d-lactate and l-lactate, with a minimal detectable concentration of approximately 5 μM. The average levels of l-lactate in plasma were 1.33 ± 0.12 mM in affected individuals and 1.43 ± 0.64 mM in healthy controls (corresponding normal levels are 0.5–2.2 mM). In urine samples, l-lactate was undetectable in both groups. We found that d-lactate levels in all affected individuals were markedly elevated in both plasma and urine. The average concentration of plasma d-lactate in affected individuals was 3.16 ± 0.63 mM and was undetectable in the healthy controls, whereas the corresponding normal levels are less than 0.043 mM (Figure 4A). In the urine samples, the average levels of d-lactate were 32.08 ± 5.77 mM in the affected individuals versus 0.65 ± 0.65 mM in the healthy controls, whereas the corresponding normal levels are 0.0–0.25 mM (Figure 4A). Thus, although we observed no evident differences in l-lactate levels between affected and healthy individuals, we detected markedly elevated levels of both plasma and urine levels of d-lactate in all affected individuals.

Excess d-lactate causes hyperuricemia in mice. To test whether hyperuricemia may be caused by elevated plasma d-lactate levels, as seen in our patients, we measured plasma uric acid levels in mice following injections of d-lactate. Naive C57BL/6 mice were injected with 200 μL PBS or 3 M d-lactate, and blood samples were taken before and at several time points after injection to monitor plasma uric acid levels. In mice injected with PBS, we observed only slight fluctuations in plasma uric acid levels over time; however, we detected a steady increase in plasma uric acid levels in mice injected with d-lactate, peaking approximately 180 minutes after injection, with an average increase in plasma uric acid levels of 54% ± 0.34% (Figure 4B).
Although urate handling in mice in particular and in mammals in general is different from that in humans, these results suggest that elevation in plasma D-lactate can cause elevations in plasma uric acid. A plausible underlying mechanism for this phenomenon could be alterations in renal handling of uric acid as a result of excess in D-lactate. This is in line with lactate being a known anti-uricosuric agent that decreases urinary clearance of uric acid by serving as a substrate for one of several renal organic anion transporters (OATs), including OAT10 and URAT1. These transporters are expressed at the apical membrane of proximal tubule cells or cortical collecting ducts, and their primary function is the inward transport of urate in exchange for the outward shift of monovalent organic anions such as lactate (25, 26); thus, excessive renal secretion of D-lactate in exchange for uric acid reabsorption could culminate in hyperuricemia. Furthermore, 2 additional renal urate transporters, OAT1 and OAT3, were recently shown to affect lactate concentrations in vitro and may therefore play a role in hyperuricemia in the presence of elevated lactate (27).

D-lactate is produced by intestinal microbes, as evidenced by D-lactic acidosis caused in the context of short bowel syndrome (28). Our data suggest the possibility that elevated levels of D-lactate could play a role in mediating the effects of nutrition and the microbiome in the pathogenesis of common forms of hyperuricemia could play a role in mediating the effects of nutrition and the alterations in renal handling of uric acid as a result of excess in D-lactate. This is in line with lactate being a known anti-uricosuric agent that decreases urinary clearance of uric acid by serving as a substrate for one of several renal organic anion transporters (OATs), including OAT10 and URAT1. These transporters are expressed at the apical membrane of proximal tubule cells or cortical collecting ducts, and their primary function is the inward transport of urate in exchange for the outward shift of monovalent organic anions such as lactate (25, 26); thus, excessive renal secretion of D-lactate in exchange for uric acid reabsorption could culminate in hyperuricemia. Furthermore, 2 additional renal urate transporters, OAT1 and OAT3, were recently shown to affect lactate concentrations in vitro and may therefore play a role in hyperuricemia in the presence of elevated lactate (27).

D-lactate is produced by intestinal microbes, as evidenced by D-lactic acidosis caused in the context of short bowel syndrome (28). Our data suggest the possibility that elevated levels of D-lactate could play a role in mediating the effects of nutrition and the microbiome in the pathogenesis of common forms of hyperuricemia could play a role in mediating the effects of nutrition and the alterations in renal handling of uric acid as a result of excess in D-lactate. This is in line with lactate being a known anti-uricosuric agent that decreases urinary clearance of uric acid by serving as a substrate for one of several renal organic anion transporters (OATs), including OAT10 and URAT1. These transporters are expressed at the apical membrane of proximal tubule cells or cortical collecting ducts, and their primary function is the inward transport of urate in exchange for the outward shift of monovalent organic anions such as lactate (25, 26); thus, excessive renal secretion of D-lactate in exchange for uric acid reabsorption could culminate in hyperuricemia. Furthermore, 2 additional renal urate transporters, OAT1 and OAT3, were recently shown to affect lactate concentrations in vitro and may therefore play a role in hyperuricemia in the presence of elevated lactate (27).

D-lactate is produced by intestinal microbes, as evidenced by D-lactic acidosis caused in the context of short bowel syndrome (28). Our data suggest the possibility that elevated levels of D-lactate could play a role in mediating the effects of nutrition and the microbiome in the pathogenesis of common forms of hyperuricemia could play a role in mediating the effects of nutrition and the alterations in renal handling of uric acid as a result of excess in D-lactate. This is in line with lactate being a known anti-uricosuric agent that decreases urinary clearance of uric acid by serving as a substrate for one of several renal organic anion transporters (OATs), including OAT10 and URAT1. These transporters are expressed at the apical membrane of proximal tubule cells or cortical collecting ducts, and their primary function is the inward transport of urate in exchange for the outward shift of monovalent organic anions such as lactate (25, 26); thus, excessive renal secretion of D-lactate in exchange for uric acid reabsorption could culminate in hyperuricemia. Furthermore, 2 additional renal urate transporters, OAT1 and OAT3, were recently shown to affect lactate concentrations in vitro and may therefore play a role in hyperuricemia in the presence of elevated lactate (27).

D-lactate is produced by intestinal microbes, as evidenced by D-lactic acidosis caused in the context of short bowel syndrome (28). Our data suggest the possibility that elevated levels of D-lactate could play a role in mediating the effects of nutrition and the microbiome in the pathogenesis of common forms of hyperuricemia could play a role in mediating the effects of nutrition and the alterations in renal handling of uric acid as a result of excess in D-lactate. This is in line with lactate being a known anti-uricosuric agent that decreases urinary clearance of uric acid by serving as a substrate for one of several renal organic anion transporters (OATs), including OAT10 and URAT1. These transporters are expressed at the apical membrane of proximal tubule cells or cortical collecting ducts, and their primary function is the inward transport of urate in exchange for the outward shift of monovalent organic anions such as lactate (25, 26); thus, excessive renal secretion of D-lactate in exchange for uric acid reabsorption could culminate in hyperuricemia. Furthermore, 2 additional renal urate transporters, OAT1 and OAT3, were recently shown to affect lactate concentrations in vitro and may therefore play a role in hyperuricemia in the presence of elevated lactate (27).
Acknowledgments

The studies were funded by the Morris Kahn Foundation and supported through the National Knowledge Center for Rare/Orphan Diseases sponsored by the Israeli Ministry of Science, Technology and Space. We thank Iftat Abramovich (Eyal Gottlieb Laboratory, Faculty of Medicine, Technion Institute of Technology, Haifa, Israel) for assistance with the mass spectrometry studies.

Address correspondence to: Ohad Birk, Genetics Institute, Soroka Medical Center, POB 151 Beer Sheva 84101, Israel. Phone: 972.8.6403439; Email: obirk@bgu.ac.il.