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ORIGINAL ARTICLE

ALPL mutations in adults with rheumatologic disorders and low serum alkaline phosphatase activity

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Abstract

Tissue-nonspecific alkaline phosphatase (ALP), encoded by *ALPL*, is important for bone homeostasis and interacts with collagen type I. In the present study, we sequenced *ALPL* and a panel of collagen type I-related genes in 24 adults (age 22–80 years; 20 female) with persistently low serum ALP (< 40 U/L) and a range of rheumatologic symptoms. We found heterozygous pathogenic or likely pathogenic variants in *ALPL* in 14 (58%) of these individuals. In addition, 7 study participants had potentially damaging heterozygous variants of uncertain significance in genes related to collagen type I. Patients who were positive for *ALPL* variants had similar age and serum ALP levels to patients in whom no *ALPL* variants were detected, but had higher serum pyridoxal-5-phosphate concentrations (median 214 nmol/L vs. 64 nmol/L; $p = 0.02$; *U* test). In summary, heterozygous *ALPL* variants are frequent in individuals with rheumatologic symptoms and low ALP serum activity. It is possible that variants in genes that are involved in collagen type I production have a modifying effect on the clinical consequences of such *ALPL* variants.

Keywords ALPL · Arthritis · Collagen type I · Hypophosphatasia · Rheumatology

Introduction

Tissue-nonspecific alkaline phosphatase (ALP) is a widely expressed cell surface enzyme that is encoded by *ALPL* [1, 2]. ALP is important for the normal development and homeostasis of bones and teeth. Hypophosphatasia (HPP), a disorder of deficient ALP activity caused by *ALPL* mutations, is characterized by bone and tooth abnormalities of wide-ranging severity. Manifestations include rickets, osteomalacia, and premature tooth loss. Most individuals with severe HPP have autosomal recessive disease; autosomal dominant forms of the disorder are also observed, especially in individuals with milder phenotypes [1, 2]. *ALPL* mutations lead to low ALP serum activity and accumulation of ALP's endogenous substrates such as pyrophosphate and pyridoxal-5-phosphate (PLP) [1]. HPP occurs worldwide but is more prevalent in several distinct populations due to their founder mutations. This is the case for the severe form of autosomal recessive HPP in the Mennonite population residing in the Canadian province of Manitoba (about 1 in 2500 newborns), due to a founder effect for the *ALPL* missense mutation p.Gly334Asp [2, 3]. Homozygosity for the *ALPL* p.Gly334Asp mutation causes classical severe perinatal/infantile HPP but heterozygous carriers of 1 copy of *ALPL* p.Gly334Asp are generally asymptomatic except for poor dentition [4–6].

Typical adult-onset HPP is characterized by frequent poorly healing stress fractures, premature loss of teeth, calcific periarthritis and ossification of ligaments as well as chronic pain [1]. It is most often caused by heterozygous *ALPL* mutations that have a dominant negative effect leading to low serum ALP levels [7]. However, only a minority of heterozygous carriers of such dominant mutations seem to develop typical signs of HPP, suggesting low penetrance [7]. On the other hand, recent studies suggest that persistently low serum ALP can be associated with unspecific musculoskeletal symptoms, such as joint pain and enthesopathies [8]. In individuals with persistently low ALP levels, heterozygous *ALPL* mutations are frequently present even if they do not present with the typical clinical picture of HPP [9–11].

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ALP interacts with collagen type I, and this interaction is influenced by the degree of post-translational glycosylation of collagen type I [12]. It is known from the study of genetic disorders affecting collagen type I, such as osteogenesis imperfecta, that the glycosylation pattern of collagen type I can be influenced by genetic variations in collagen type I encoding genes, as well as by many other genes that are involved in the post-translational modification of collagen type I [13–15]. We, therefore, hypothesized that variants in such collagen type I-related genes might contribute to the phenotypic variability associated with heterozygous *ALPL* variants.

In the present study, we identified 24 individuals who were referred to the adult metabolic genetics clinic in Winnipeg by rheumatologists, endocrinologists, and hepatologists in Winnipeg, Manitoba, and who had persistently low serum levels of ALP. Sequence analysis of *ALPL* and of a gene panel related to collagen type I was performed in all individuals.

Materials and methods

Subjects

The study population was comprised of individuals who were referred to the adult metabolic clinic at the Health Sciences Centre, a tertiary care facility affiliated with the Winnipeg Regional Health Authority, from subspecialty rheumatology, endocrinology or hepatology clinics and who had persistently low serum levels of ALP (< 40 U/l). The study was approved by the Institutional Review Board of the University of Manitoba. All participants provided informed consent.

Sequence analysis

Sequence analysis of a metabolic bone disease gene panel was performed using an Ion Torrent PGM device (Life Technologies), as described [16]. Apart from *ALPL* (NM_000478.4), the following genes were analyzed: *COL1A1* (NM_000088.3), *COL1A2* (NM_000089.3), *BMP1* (NM_001199.3, NM_006129.4), *CRTAP* (NM_006371.4), *FKBP10* (NM_021939.3), *IFITM5* (NM_001025295.2), *P3H1* (NM_022356.3), *PLOD2* (NM_000935.2), *PPIB* (NM_000942.4), *SERPINF1* (NM_002615.5), *SERPINH1* (NM_001235.3), *SP7* (NM_152860.1), *TMEM38B* (NM_018112.2), *CREB3L1* (NM_052854.3), *P4HB* (NM_000918.3), *PLS3* (NM_001136025.4), *RUNX2* (NM_001024630.3), *SEC24D* (NM_014822.2), *SPARC* (NM_003118.3), *WNT1* (NM_005430.3), *XYLT2* (NM_022167.3).

A total of 10 ng DNA per sample was used for target enrichment by multiplex PCR and sequencing on an Ion 316 Chip (Ion PGM Hi-Q Sequencing Kit; Life Technologies), which was performed following the manufacturer's instructions with the 200-bp single-end run configuration. Data from the sequencing run were processed using Torrent Suite software (version 5.04; Life Technologies) for base calls, read alignments, and variant calling using the reference genomic sequence (hg19) of target genes. Variants were not confirmed by Sanger sequencing, as our prior results indicated that the sequencing methodology used in this study is at least as sensitive as Sanger sequencing [17]. We, therefore, do no longer perform Sanger confirmation on a routine basis. Called variants were annotated using Ion Reporter (version 5.0) that provides information on whether variants are predicted to be damaging (based on SIFT [18] and PolyPhen-2 [19]) and whether they are conserved in evolution (based on PhyloP [20]). All sequence changes were compared to their frequency in the Exome Aggregation Consortium (ExAc) database [21]. The standards and guidelines of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology were used to classify variants as pathogenic or likely pathogenic [22].

Statistical analyses

Differences in medians between two groups were tested for significance using the Mann–Whitney *U* test. Variant prevalence was compared between groups using the Chi square test. All tests were two-tailed and throughout the study p values < 0.05 were considered significant. These calculations were performed using the SPSS Statistics software version 23.0 (SPSS Inc., Chicago, Illinois, USA).

Results

The study population was comprised of 24 individuals (20 female, 4 male) aged 22–80 years (median 49 years) (Table 1). The clinical symptoms and diagnoses varied widely and often included diffuse joint and bone pain. Three patients were diagnosed with osteoarthritis, two had a diagnosis of rheumatoid arthritis, one with Castleman disease and one with erosive arthropathy and two had a history of recurrent fractures. Premature loss of teeth was noted in one individual. There was no history of rickets or bowed legs in childhood, delayed motor milestones, frequent fractures, craniosynostosis or seizures. None had a family history of confirmed HPP. None had known other causes of low ALP such as low magnesium, malnutrition, cardiac failure, hypothyroidism or coeliac disease. As per study design, all study participants had serum ALP levels that were persistently

Table 1 Clinical characteristics and sequencing results in individuals with pathogenic (**a**) and without pathogenic or likely pathogenic (**b**) *ALPL* variant

Sex	Age	Ethnicity	ALP (U/L)	PLP (nmol/L)	*ALPL* change	VUS	Diagnosis/symptoms
(a)							
F	23	Mennonite	32	94	p.Val95Met	None	Intermittent toe joint inflammation; restless arms; occasional stiff fingers
F	49	Mennonite	36	148	p.Val95Met	None	Osteoarthritis; degenerative back pain
F	54	Ukrainian	6	53	p.Thr134His	None	Bone pain lower legs; fracture right 5th metatarsal
F	67	Unknown	6	149	p.Asp294Ala	None	Osteoarthritis; bilateral knee replacements; pain in hands and other joints
M	51	Filipino	19	149	p.Gln361Argfs*45	None	Fatigue; weakness
M	60	Mennonite	18	278	p.Gly334Asp	None	Joint pain (back, shoulder, knees); torn rotator cuff muscles; Dupuytren's contractures of distal joints of the 5th fingers bilaterally
F	43	Mennonite, Ukrainian	17	883	p.Gly334Asp	SEC24D: p.Leu526Phe	Reactive arthropathy; osteoarthritis; fractures in toes and fingers; broken teeth within last 9 years
F	59	Mennonite	26		p.Gly334Asp	None	Joint pain; swelling of left thumb, left shoulder, both knees and spine; bone pain in both feet
M	44	Mennonite	10		p.Gly334Asp	COL1A2: p.Ala792Asp	Pain in hands, shoulders and hips
F	33	Mennonite	27	439	p.Gly334Asp	BMP1: p.Arg371His LRP5: p.Arg746Trp	Chronic diffuse joint pain
F	39	Mennonite	27	664	p.Gly334Asp	None	Aching and painful joints (back, shoulder, knees); both shoulders surgically repaired due to torn rotator cuff muscles; Dupuytren's contractures of distal joints of the 5th fingers bilaterally
F	63	Mennonite	6	434	p.Gly334Asp	COL1A2: p.Gln1293His BMP1: p.Ser692Phe	Fibromyalgia; osteoporosis; arthritis; chronic lower back pain
F	49	Mennonite	16		p.Gly334Asp	P4HB: p.Val28Met	Rotator cuff injury; leg cramps with fatigue; 'crumbly' adult teeth
F	36	Mennonite			p.Gly334Asp	None	Foot pain; inflammatory changes in thumbs; plantar fasciitis
(b)							
M	67	Mennonite	29	156	None	None	Hypothyroidism; pain in both knees
F	68	Polish, Ukrainian	25	64	None	None	Castleman Disease; connective tissue disorder; pain in thighs; intermittent tingling and numbness in lower limbs

Table 1 (continued)

Sex	Age	Ethnicity	ALP (U/L)	PLP (nmol/L)	*ALPL* change	VUS	Diagnosis/symptoms
F	46	Filipino	27	99	None	COL1A1: p.Arg564Cys PLOD2: p.Arg680Gln	Rheumatoid arthritis
F	66	Polish, German	24	127	None	None	Polymyalgia rheumatica; vasculitis; arthropathy
F	51	Australian, Ukrainian, German	17	48	None	SEC24D: p.Leu526Phe	Rheumatoid arthritis involving mostly hands and feet; numerous cavities
F	48	French-Canadian, Polish, Scottish	30	52	None	None	Hypothyroidism; muscle and joint pain; fatigue; leg and foot cramping; history of lupus
F	40	Mennonite, Irish/Scottish	28		None	None	Arthritis; bilateral patellofemoral pain; bilateral tendinosis; numerous fractures (~30 in legs, arms, hands, fingers)
F	81	Scottish, English	29	274	None	None	Discoid lupus; tingling in feet
F	42	Mennonite, German	31	51	None	None	Swollen right 3rd metacarpo-phalangeal joint; pain in feet; difficulty walking at end of day
F	41	Ukrainian	13	63	None	None	Muscle weakness; sore joints; extreme fatigue; hypothyroidism; premature loss of teeth

Reference ranges ALP: 40–120 U/L; PLP 20–96 nmol/L

VUS variants of uncertain significance

below 40 U/L. At the time of the investigation, serum ALP ranged from 6 to 36 U/L. Serum PLP concentrations, measured in 19 individuals, ranged from 48 to 883 nmol/L (reference range 20–96 nmol/L).

Sequence analysis of genomic DNA revealed pathogenic or likely pathogenic *ALPL* variants (all heterozygous) in 14 of the 24 individuals (58%) (Table 1). *ALPL* variants were detected in 3 of the 4 male and in 11 of the 20 female patients included in this study. Of the 5 different *ALPL* variants that were found in this study population, 4 were missense variants and one introduced a frameshift (Table 2). Three of the missense variants had previously been reported as pathogenic [3, 23, 24]. The most frequently observed variant in our study population was p.Gly334Asp, present in 9 study participants. The other *ALPL* variants were each observed in 1 or 2 individuals.

Patients who were positive for *ALPL* variants had similar age and serum ALP levels to patients in whom no *ALPL* variants were detected. However, serum PLP concentrations were significantly higher in the group with *ALPL* variants (median 214 nmol/L vs. 64 nmol/L; $p = 0.02$; *U* test).

In addition to *ALPL* variants, sequence analysis revealed 9 different variants of uncertain significance (VUS) in other genes that were predicted to have a detrimental effect on protein function (Table 3). Five of these VUS were found in genes that are associated with dominant bone disorders, 4 VUS were present in recessive genes. Overall, VUS were identified in 7 study participants, of whom 5 also had a *ALPL* variant (p.Gly334Asp in each case) (Table 1). Thus, 5 of 9 individuals (56%) with the p.Gly334Asp mutation, but only 2 of the other 15 study participants (13%) had a VUS in genes related to collagen type I production ($p < 0.001$ by Chi square test).

Discussion

In this study, we found that 14 of 24 (58%) individuals with rheumatologic disorders and persistently low ALP activity had heterozygous pathogenic or likely pathogenic variants in *ALPL*. The p.Gly334Asp mutation was the most prevalent *ALPL* variant, affecting 9 individuals. In addition, potentially damaging heterozygous VUS in genes related to collagen type I production were found in 7 patients, 5 of whom were also heterozygous for the p.Gly334Asp variant in *ALPL*.

Our observation that 58% of our study participants had detectable *ALPL* variants is in accordance with recent sequencing studies on clinical cohorts with low ALP. A

Table 2 Rare *ALPL* variants found in the study population

Amino acid change	Nucleotide change	Allele frequency[a] (%)	SIFT	PolyPhen2	PhyloP (100 way)	Previously described in HPP	Functional consequence
p.Val95Met	c.283G > A	0.004	0.11	0.92	6.45	No	Unknown; located in homodimeric interface [27]
p.Thr134His	c.400_401AC > CA	0	0	1.00		In perinatal HPP [23]	Unknown
p.Asp294Ala	c.881A > C	0.004	0.01		8.83	In infantile HPP [24]	35% residual activity [28]
p.Gly334Asp	c.1001G > A	0	0.001	1.00	9.93	In perinatal HPP [3]	2% residual activity (6); mild dominant negative effect [7]
p.Gln361Argfs*45	c.1078_1081dup	0.0004				Similar frameshift (1083_1084dup, p.Ser364Argfs*42) published as disease-causing [29]; homozygous frameshift mutations are an established cause of severe HPP	Likely nonsense mediated decay on mRNA level

[a]According to ExAc browser

Table 3 Variants of uncertain significance in genes related to bone fragility disorders

Gene	Amino acid change	Nucleotide change	Allele frequency[a] (%)	SIFT	PolyPhen2	PhyloP (100 way)
Dominant						
COL1A1	p.Arg564Cys	c.1690C > T	0	0	1.0	4.19
COL1A2	p.Ala792Asp	c.2375C > A	0	0.07	0.52	4.11
COL1A2	p.Gln1293His	c.3879G > T	0	0.02	1.0	1.65
LRP5	p.Arg746Trp	c.2236C > T	0.004	0.001	1.0	0.81
P4HB	p.Val28Met	c.82G > A	0	0	1.0	5.32
Recessive						
BMP1	p.Arg371His	c.1112G > A	0.37	0.02	0.99	3.05
BMP1	p.Ser692Phe	c.2075C > T	0.02	0.004	1.0	7.91
PLOD2	p.Arg680Gln	c.2039G > A	0.005	0.01	0.93	3.22
SEC24D	p.Leu526Phe	c.1576C > T	0.9	0.05	1.0	9.92

[a]According to ExAc browser

study in the United States found *ALPL* mutations in 42 of 50 individuals (84%) with ALP below 30 U/L [9], whereas investigators in Spain detected *ALPL* mutations in 36 of 83 (43%) individuals with low ALP [11].

In the past, *ALPL* sequencing studies were mostly limited to patients with typical signs of HPP, and a pathogenic mutation could be found in most such individuals [25]. Studies, such as the present one, that recruited participants on the basis of persistently low ALP levels rather than typical clinical signs of HPP indicate that *ALPL* mutations are often associated with non-specific symptoms, such as diffuse musculoskeletal pain and arthritic complaints [9, 11]. Accordingly, only a small minority of our study participants had typical manifestations of HPP, such as fractures, pseudofractures or tooth loss.

ALP interacts with collagen type I [12] and it has been suggested that genetic variants in collagen type I may modify the effect of *ALPL* variants [26]. We, therefore, investigated our study population for the presence of variants that may modify collagen type I production. Even though the functional importance of the identified VUS has not been established, they were all rare and were predicted to have a damaging effect on protein function. Carriers of the p.Gly334Asp mutation were significantly more likely to have a VUS in one of the investigated genes than other study participants. Among study participants with

ALPL variants, VUS were found only in individuals carrying the p.Gly334Asp mutation. This may indicate that the presence of variants in other genes makes carriers of the *ALPL* p.Gly334Asp mutation more likely to develop symptoms. The sample size of the present study was too small to arrive at definite conclusions, but the effect of collagen type I-related genes on the consequences of *ALPL* variants merits further investigation.

In summary, we found that 58% of individuals with rheumatologic symptoms and low ALP serum activity had heterozygous *ALPL* variants. It is possible that variants in genes that are involved in collagen type I production have a modifying effect on the clinical consequences of such *ALPL* variants.

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Web resources The Tissue Nonspecific Alkaline Phosphatase Gene Mutations Database: http://www.sesep.uvsq.fr/03_hypo_mutations.php. Exome Aggregation Consortium (ExAC) Browser: http://exac.broadinstitute.org/. Online Mendelian Inheritance in Man (OMIM), http://www.omim.org.

Author contributions FR wrote the manuscript; GB performed sequence analyses; CRG conceptualized the project, contributed patient information, finalized the report and accepts responsibility for the integrity of the data analysis. All authors have read and approved of the final version of the manuscript.

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Compliance with ethical standards

Conflict of interest Frank Rauch has received consultancy fees from Alexion Inc. Ghalib Bardai declares no conflict of interest. Cheryl Rockman-Greenberg was principal investigator of Alexion-sponsored clinical trials of enzyme replacement therapy for the treatment of HPP, is a member of the Scientific Advisory Board of the Alexion-sponsored International HPP Registry and has received honoraria for Alexion-sponsored webinar and symposia.

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