Potential pathological role of single nucleotide polymorphism (c.787T>C) in alkaline phosphatase (ALPL) for the phenotypes of hypophosphatasia

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Abstract. Hypophosphatasia (HPP; OMIM 241510, 241500, and 146300) is an inherited metabolic disease characterized by defects of bone and tooth mineralization, which is caused by loss-of-function mutations in the ALPL gene encoding tissue non-specific alkaline phosphatase (TNSALP). In the last three decades, several studies have focused on the genotype-phenotype correlation in hypophosphatasia (HPP). In particular, functional tests based on in vitro analysis for the residual enzymatic activities of mutations have revealed a clear but imperfect genotype-phenotype correlation, suggesting that multiple potential factors modulate the phenotype. One of the missense variants identified in the tissue non-specific alkaline phosphatase (ALPL) gene, c.787T>C, has been considered as a benign polymorphism in HPP; however, its pathogenicity and role in disease manifestation remain controversial. We here report our recent experience of three unrelated families harboring the c.787T>C variant, suggesting clinical implications regarding the controversial pathogenicity of c.787T>C. First, despite the lack of obvious clinical phenotypes, homozygous c.787T>C would decrease the serum level of ALP activity. Second, c.787T>C might deteriorate phenotypes of a patient harboring another ALPL variant, especially one that has thus far presumed to be benign, e.g., the c.1144G>A variant. These cases contribute to the recent advances in understanding HPP to facilitate clinical recognition of more subtle phenotypes, further providing insights into the pathogenesis of HPP.

Key words: Hypophosphatasia, Tissue-nonspecific alkaline phosphatase (TNSALP), alkaline phosphatase (ALPL) gene, Single nucleotide polymorphism (SNP)

HYPOPHOSPHATASIA (HPP; OMIM 241510, 241500, and 146300) is an inherited metabolic disease characterized by defects of bone and tooth mineralization, which is caused by loss-of-function mutations in the ALPL gene encoding tissue non-specific alkaline phosphatase (TNSALP) [1]. The clinical spectrum of HPP extends over a wide range from only affecting the teeth, i.e., odontohypophosphatasia, to severe skeletal abnormalities that could be fetal lethal. Thus, the signs and symptoms of HPP can appear at any period of life from before birth to adulthood, although more severe forms tend to occur before birth and in early infancy. To date, more than 300 mutations of ALPL have been identified, and the disease has been shown to be inherited in either an autosomal recessive or autosomal dominant manner.

Several studies conducted over the past three decades have revealed clear genotype-phenotype correlations in HPP, which are largely been based on functional tests with in vitro analysis for determining the residual enzymatic activities of these mutations in ALPL [1]. These findings suggest that multiple potential factors, including environmental and genetic backgrounds, modulate the
phenotype. One of the missense variants in ALPL, c.787T>C, has been considered to be a benign polymorphism [2], although there is still controversy surrounding its specific effects and contribution to disease development and manifestation [3]. We here report the clinical findings of subjects with the c.787T>C variant from unrelated three families, suggesting that the variant can have a pathological role in HPP, but that its effect may be dependent on the environmental and genetic background.

**Clinical Report**

The pedigrees and clinical characteristics of the three families including c.787T>C carriers are summarized in Fig. 1 and Table 1, respectively.

**Family 1**

The proband of Family 1 (I-II-2) was a 3.5-year-old boy with short stature (−2.0 SD) and low serum alkaline phosphatase (ALP) (JSCC method) activity of 280 U/L (−2.7 SD) determined during routine blood examination [4]. The patient had no skeletal or tooth abnormality, but urinary phosphoethanolamine (PEA) (HPLC method) levels were elevated at 220 nmol/mgCr (reference range: 31–110 nmol/mgCr), which is consistent with HPP. The father (I-I-1) had a history of vertebral fracture and also exhibited low serum ALP activity at 100 U/L (−2.6 SD). The mother and younger brother had low ALP and increased urinary PEA levels but without any other symptoms of HPP.

**Family 2**

At the age of 18 years, the proband of Family 2 (2-II-1) was referred to our hospital due to afebrile convulsion. Routine examination revealed low serum ALP activity of 73 U/L (−2.8 SD) without hypocalcemia. The

![Fig. 1](image_url) Pedigree charts of Family 1, and Family 2 in the present study. The probands are indicated by arrows. Black and hatched line indicate c.787T>C and c.1559delT, respectively.

| Table 1 |  |
|---|---|
| **Family** | **Sex** | **Age** | **Genotype** | **Height SD** | **ALP (U/L)*** | **ALP SD** | **PEA (μmol/g Cr)** | **Notes** |
| 1 | I-1 | M | 39 yrs | c.787T>C | 0 SD | 100 | −2.6 SD | N.D | Fracture |
| | I-2 | F | 39 yrs | c.787T>C | −0.8 SD | 90 | −2.3 SD | 140 |
| | II-1 | F | 6 y 5 m | N.D | −1.0 SD | 658 | −1.0 SD | N.D |
| | II-2 | M | 3 y 6 m | c.787T>C | −2.0 SD | 280 | −2.7 SD | 220 | Short stature |
| | II-3 | M | 11 months | c.787T>C | −1.5 SD | 404 | −2.0 SD | 180 |
| 2 | I-1 | M | 52 yrs | N.D | −0.13 SD | N.D | N.D | N.D |
| | I-2 | F | 46 yrs | c.787T>C | −0.39 SD | 80 | −2.6 SD | N.D | Fracture |
| | II-1 | F | 19 yrs | c.787T>C | −1.5 SD | 73 | −2.8 SD | 205 | Seizure |
| | II-2 | M | 11 yrs | c.1559delT | −0.5 SD | 506 | −1.2 SD | 108 |
| 3 | II-2 | F | 2 y 8 m | c.787T>C | +1.7 SD | 273 | −2.8 SD | 581.3 | Early shedding of baby teeth |

*: ALP was assayed by JSCC (Japan. Society of. Clinical. Chemistry) method

**: SD value was calculated according to the data of the Japanese normal reference data [4]
patient did not show any skeletal or tooth abnormality, but urinary PEA levels were increased at 205 nmol/mgCr. Furthermore, the serum level of the TNSALP substrate pyridoxal phosphate and the pyridoxal-phosphate/pyridoxal ratio (PLP/PL), which was measured using high-performance liquid chromatography with fluorescence detection, were elevated at 320.6 nmol/L (reference range: 20.5–151 nmol/L) and 12.4 (reference range: 1.0–4.2), respectively, suggesting impaired TNSALP activity [5-7]. The mother (2-I-2) had a history of frequent fractures and demonstrated low ALP at 80 U/L (–2.7 SD). However, the ALP activity of the younger brother (2-II-2) was normal (506 U/L; –1.2 SD).

Family 3
At age of 2 years and 8 months old, a girl of family 3 developed premature shedding of the primary teeth. The patient had low serum ALP activity at 273 U/L (–2.8 SD) and no family history of HPP symptoms. Despite of absence of bone deformities or a short stature, the urinary PEA level was elevated at 581.3 nmol/mgCr.

Mutation analysis
Written informed consent for DNA analysis was obtained from the parents. All procedures performed in studies were in accordance with the ethical standards of the institutional and/or national research committee (the Research Ethics Committee of Osaka University) and with the 1964 Helsinki declaration including its later amendments or comparable ethical standards. Genomic DNA was extracted from peripheral white blood cells, and the entire coding region and exon-intron boundaries of the ALPL gene were amplified with specific primers (Table 2). Direct sequencing in both directions was performed on an Applied Biosystems 3730 Genetic Analyzer.

Screening for mutations in the ALPL gene revealed the c.787T>C polymorphism (rs3200254) along with previously reported mutations, c.1559delT (p.Leu520ArgfsX86) or c.1144G>A (p.Val382Ile), in the three probands. In family 1, the proband (1-II-2) was compound heterozygous of c.787T>C and c.1559delT that were inherited from his mother (1-I-2) and his father (1-I-1), respectively. In family 2, variant of c.787T>C/c.1559delT was identified in the proband (2-II-1). The same compound heterozygote variant of c.787T>C/c.1559delT was also found in his mother (2-I-2) and c.1144G>A was identified. Familial analysis was not performed in family 3.

Discussion
Based on our findings of these three families, we propose two clinical implications regarding the controversial pathogenicity of c.787T>C. First, despite the lack of obvious clinical phenotypes, homozygous c.787T>C would decrease the serum level of ALP activity. Second, c.787T>C might deteriorate phenotypes of a patient harboring another ALPL variant, especially one that has thus far presumed to be benign.

The c.787T>C in exon 7 of ALPL leads to a single amino acid substitution, p.Tyr263His. Based on the 3D modeling, the dimer structures formed by two Wild-type TNALP proteins and the combination of Wild-type/mutant (c.787T>C) TNALP proteins were constructed based on the mRNA data of alkaline phosphatase, tissue-nonspecific isozyme isoform 1 preproprotein (NM_000478.6) using the COTH algorithm (https://zhanglab.ccmb.med.umich.edu/COTH/) [8]. These models were visualized using the PyMOL system (https://pymol.org/2/) (Fig. 2).

Table 2 Primer sets for ALPL gene analysis

| ALP2F      | AATAAAGTGAGTGAAAGAGAT |
| ALP2R      | TTTATGCCCTCCACTGTGTCCC |
| ALP3F      | TTACCAGGCAAGTGACTGC   |
| ALP3R      | CTAGGTGTCCTGGTCACCC   |
| ALP4F      | GAGAGCAGGACGACAGAGAGA |
| ALP4R      | CTCAAGCTGGATCTGAGAT   |
| ALP5F      | GCCAGCCTGGCACTGGGCGAG |
| ALP5R      | TGAAAGCTGAGGCTGACAGAG |
| ALP6F      | ACACCAGGAGTCTCGAGATAAA |
| ALP6R      | GCCAGCCTGGCACTGGGCGAG |
| ALP7F      | TGGAAACCGCTCGAGAAGTGTAG |
| ALP7R      | CTCAAGGCACATGGGACAGCAG |
| ALP8F      | TAGCCCCCGGCATGTGCTGAC |
| ALP8R      | GGGCTCTGCCCCAGTGGCTG |
| ALP9F      | TTCCTGGAGCTCTCTAGGAC |
| ALP9R      | TGGACTCTCCATCTGCAACCC |
| ALP10F     | TCCCTCCTCCCCTACCCAGG |
| ALP10R     | GGAGACCCAGGAGGTTGGCAC |
| ALP11F     | CTGGGAGACTGCTACTCCCTG |
| ALP11R     | CCAACGCCATCCCCAGAGGGGT |
| ALP12F     | CTGGAGGAGGAGAGTGGAAAGC |
| ALP12R     | TGGCATCTGTCACCGGCTTGT |

SNP in TNALP affect hypophosphatasia

TNALP three-dimensional modeling

Three-dimensional models of the dimer structures formed by two Wild-type TNALP proteins and the combination of Wild-type/mutant (c.787T>C) TNALP proteins were constructed based on the mRNA data of alkaline phosphatase, tissue-nonspecific isozyme isoform 1 preproprotein (NM_000478.6) using the COTH algorithm (https://zhanglab.ccmb.med.umich.edu/COTH/) [8]. These models were visualized using the PyMOL system (https://pymol.org/2/) (Fig. 2).
analysis, p.Tyr263 was located on the Ca\textsuperscript{2+} binding domain, distant from the interacting domain for dimerization (Fig. 2), suggesting that p.Tyr263 may have a role in Ca\textsuperscript{2+} binding, but not in dimerization [9]. The c.787T>C was previously described as a polymorphism, because the frequencies of the variant in the normal and affected populations with hypophosphatasia were not significantly different [2]. Further, the allele frequency of the variant in the general population is higher than that of the prevalence in HPP patients. Specifically, the minor allele frequency is reported to be 0.1833 in the gnomAD database (rs3200254 in the dbSNP at http://www.ncbi.nlm.nih.gov/snp/rs3200254) and in the Japanese population, the frequency appears to be higher at 0.31–0.55 [10]. Consistently, the differences of serum ALP activities between subjects homozygous for the c.787T>C variant and healthy subjects [11] were not significant, in a previous study of Japanese young adults.

However, several reports suggested that the c.787T>C polymorphism might influence the clinical phenotypes of HPP [2]. A functional in vitro assay demonstrated that the Km value for TNSALP in cells harboring c.787T>C was decreased [10]. The c.787T>C also decreased ALP activity in a dose-dependent manner and is not likely to have dominant negative effects [12]. Clinically, c.787T>C has been reported to be potentially involved in bone metabolism, such as reducing bone mineral density in older postmenopausal women and the levels of serum bone-specific ALP in the young Japanese population [10, 11].

In the present cases, among the subjects in Family 1 with homozygous c.787T>C, the younger brother of the proband (1-II-3) and his mother (1-I-2) had low serum ALP activity with increased urinary PEA levels. We speculate that the homozygous variant of c.787T>C can have a pathological role in HPP, but that the effect may be dependent on environmental factors. Consistently, in the two cases, no clinical phenotypes, such as skeletal abnormalities, hypercalcemia, premature loss of primary teeth, short stature, and recurrent fractures, were documented, suggesting that low ALP levels might be temporary rather than permanent. Furthermore, the calcium requirement of the two cases were presumably increased due to body growth during the phase from childhood to adolescence (1-II-3) and a breastfeeding (1-I-2) [13]. Under those conditions, the homozygous variant of c.787T>C would be more likely to decrease the level of serum ALP activity.

Despite the laboratory data and the genotypes of the parents being missing, we assume that the 3-II-2 phenotype was, at least partially, caused by the c.787T>C (p.Tyr263His) homozygous variant and that the impact of the c.1144G>A (p.Val382Ile) heterozygote variant would be limited. An in vitro analysis revealed that the c.1144G>A (p.Val382Ile) variant severely impaired enzymatic activity with a dominant negative effect [14]. However, in vivo, the homozygous variant of c.1144G>A (p.Val382Ile) only caused a mild phenotype, a perinatal benign form of HPP [15], suggesting that the impact of the heterozygous c.1144G>A (p.Val382Ile) variant would be extremely limited. Consistently, both parents of 3-II-2, one of which expected to carry the heterozygous c.1144G>A (p.Val382Ile) variant, did not show any phenotype or history of HPP.

Based on our observation, c.787T>C would be expected to affect the phenotypes of patients with a heterozygous variant presumed to be benign, such as c.1559delT. Most heterozygous c.1559delT carriers have been reported to show normal levels of both ALP activity and urinary PEA [16], and the c.1559delT variant is the most frequent variant detected in the Japanese population [17]. Indeed, the serum ALP activity in the family member heterozygous for c.1559delT (2-II-2) was mildly but not pathologically low. By contrast, ALP activity in the subjects with compound heterozygote variants of c.787T>C and c.1559delT (1-I-1, 1-II-2, and 2-II-1) was
pathologically low, falling below −2 SD for respective age-matched controls. Moreover, subjects with the compound heterozygote variants of c.787T>C and c.1559delT also demonstrated HPP-like symptoms (2-I-2), including bone fracture, short stature, and afebrile convulsion.

The recent better understanding of HPP pathogenesis and manifestations has enabled the clearer recognition of subtle clinical phenotypes [18, 19]. This has been accompanied by an increased rate of HPP diagnosis, including those of milder phenotypes, suggesting a higher incidence than previously estimated. Accordingly, ALPL variants that were previously considered benign have now been linked to HPP with milder phenotypes or with lower penetrance. A sequential genome-wide association study indicated that ALPL variants are also more prevalent than previously estimated [18], and the variant c.787T>C appears to be a representative example of this situation.

Nevertheless, currently the genotype-phenotype correlations in HPP remains unclear and imperfect. Indeed, in contrast to the other members of the Family 1, the ALP level was not reduced in 1-II-1, whose predicted genotype from the parents’ information were either homozygote c.787T>C variant or the c.787T>C and c.1559delT compound heterozygote variants. Although ethical issues did not allow us to analyze the genotype of 1-II-1, multiple potential factors, including unidentified environmental and/or genetic backgrounds would have modulated the phenotypes.

It is beyond the scope of the present study to identify the factors that influence the relationships between genotype and phenotype. We presume that the required phosphatase activity might differ according to the life stage. Therefore collecting precise longitudinal data of the patients is essential. Further, epigenetic modifications and variants in non coding sequences may also affect transcriptional activity of TNSALP. To understand the roles of those factors, massive genetic study with novel approaches, such as whole genome sequence (WGS), whole genome bisulfite sequencing (WGBS) and transcriptome analysis, are required.

Because of its high prevalence and no significant difference of ALP level between subjects homozygous for the c.787T>C variant and healthy subjects, the variant c.787T>C in ALPL has been considered to be a benign polymorphism. Despite the limitations of this study, including the small number of cases, a descriptive nature without precise functional analysis in vivo or in vitro, our findings still suggest the potential pathogenicity of the c.787T>C polymorphism, along with a potential pathological role of the variants c.1559delT and c.1144G>A. Further, we presume that the susceptibility for c.787T>C variant may change according to the life stage. To resolve the controversy related to the pathogenicity of variants that are currently considered benign, further accumulation of HPP cases and genotypes would help and shed light on the pathological mechanisms of HPP.

**Author Contributions**

NM, KT, TM, KK, KO conceived the project. NM, ST, EO, YO treated the patient and collected clinical data. Gene analysis was performed by TK, YO and MF. YI carried out 3D structure model analysis. NM, KT, and KK wrote the manuscript, and KO critically reviewed the manuscript. All authors approved the manuscript.

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