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(54) Title: TREATING MUSCLE WEAKNESS WITH ALKALINE PHOSPHATASES

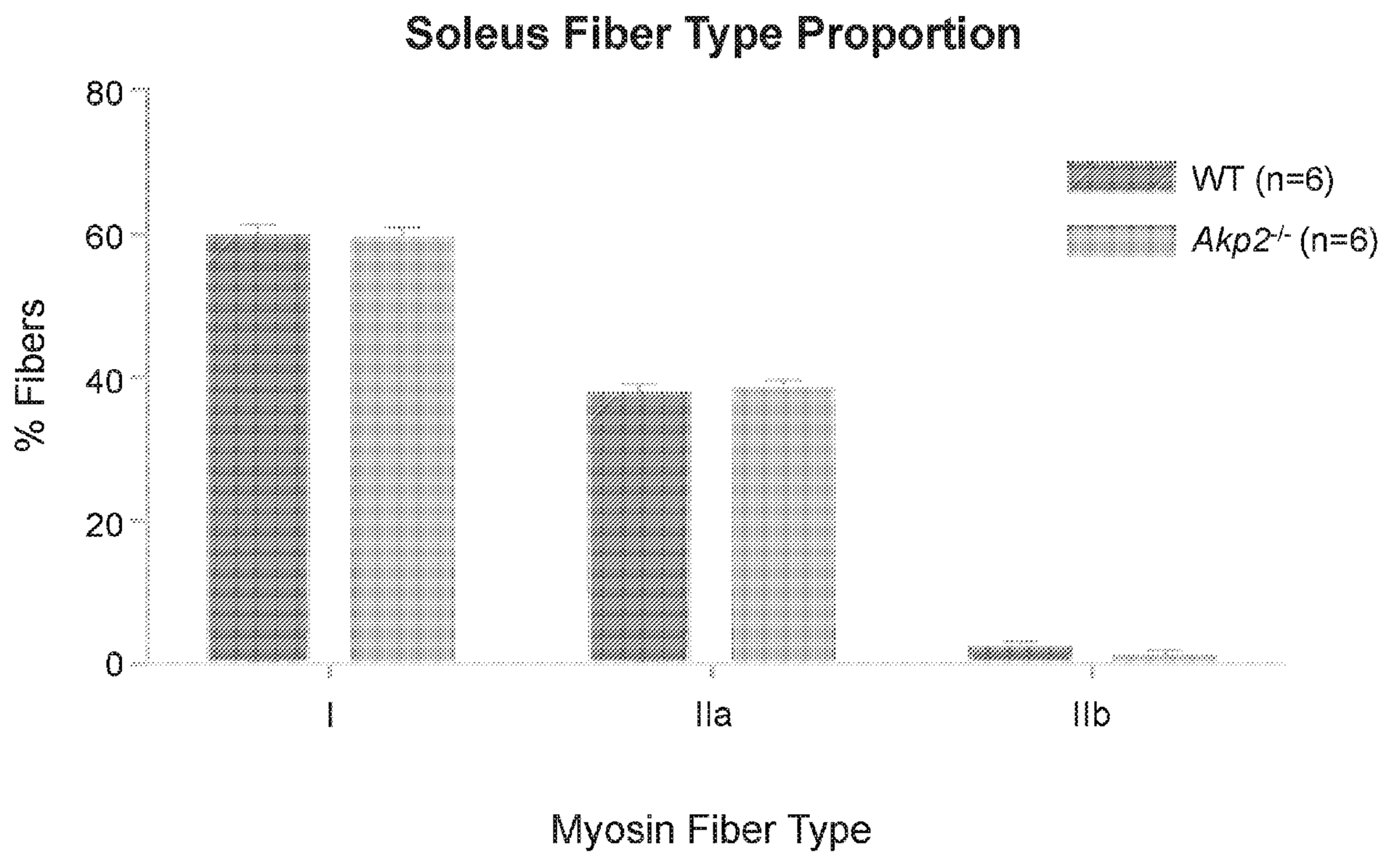

FIG. 1

(57) Abrégé/Abstract:
The disclosure features methods for treating or ameliorating at least one symptom of a subject having or being prone to a muscle weakness disease, comprising administering to said subject a therapeutically effective amount of at least one recombinant polypeptide having alkaline phosphatase activity.

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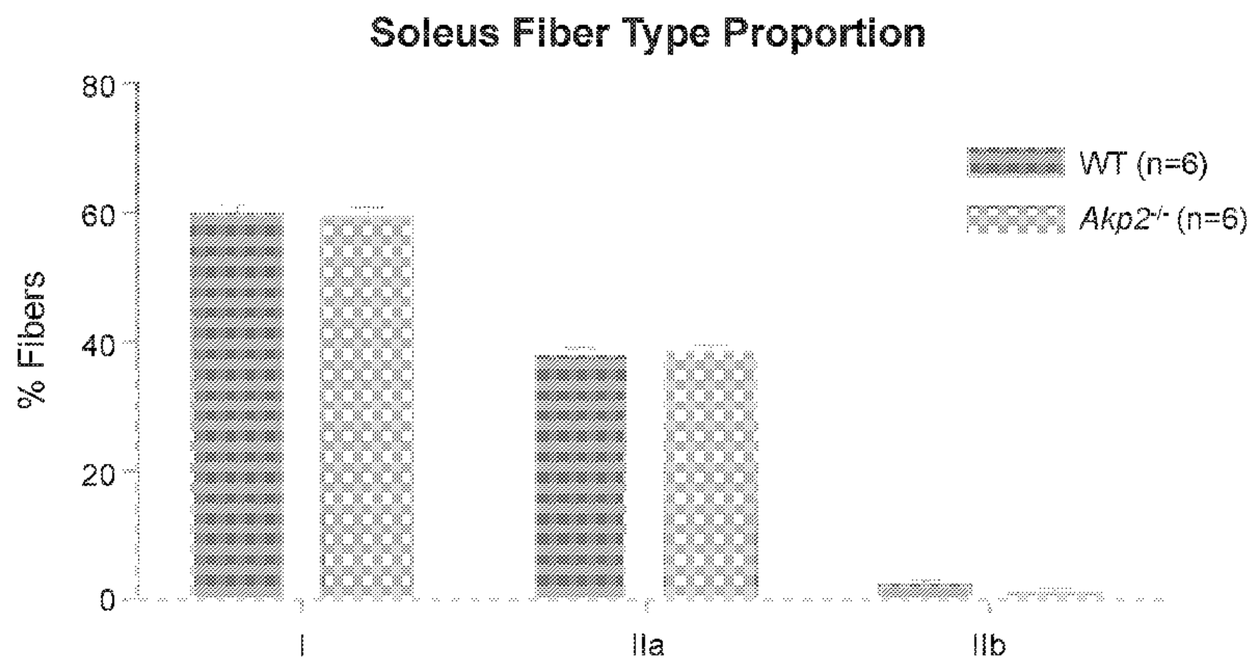

FIG. 1

(57) Abstract: The disclosure features methods for treating or ameliorating at least one symptom of a subject having or being prone to a muscle weakness disease, comprising administering to said subject a therapeutically effective amount of at least one recombinant polypeptide having alkaline phosphatase activity.

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TREATING MUSCLE WEAKNESS WITH ALKALINE PHOSPHATASES

SEQUENCE LISTING

The amino acid sequences listed in the accompanying sequence listing are shown using standard three-letter code for amino acids, as defined in 37 C.F.R. 1.822. The Sequence Listing is submitted as an ASCII text file, created on March 30, 2017, about 84 KB, which is incorporated by reference herein.

BACKGROUND

Hypophosphatasia (HPP) is a rare, heritable skeletal disease with an incidence of 1 per 100,000 births for the most severe forms of the disease. HPP is often fatal when observed at birth, having an infant mortality rate of ~70%. Severely affected patients often die in infancy from respiratory insufficiency due to progressive chest deformity.

HPP can result from loss-of-function mutations in the gene coding for tissue-nonspecific alkaline phosphatase (TNALP). HPP leads to a remarkable range of symptoms and severity, from rickets (osteomalacia) to almost complete absence of bone mineralization *in utero*. Most patients exhibit the characteristics of skeletal changes, short stature, painful lower limbs, gait disturbance, and premature shedding of teeth. For instance, infantile symptoms of HPP can include inadequate weight gain, the appearance of rickets, impaired skeletal mineralization, progressive skeletal demineralization, rib fractures, and chest deformity, while childhood symptoms of HPP can include short stature and skeletal deformities, such as bowed legs and enlarged wrists, knees, and ankles as a result of flared metaphyses. Muscle weakness (or hypotonia) is also an important symptom associated with HPP. Due to physical impairments associated with HPP, patients afflicted with HPP often exhibit a decreased ability or inability to perform routine activities that healthy patients perform on a daily basis without requiring assistance.

Hypotonia in HPP has been asserted, without data, to be a result of PPi toxicity (Whyte, M.; *J. Bone Mineral Res.* (Jan. 2017)). One paper showed PPi was able to disrupt actin/myosin interactions in a bovine muscle model (*Meat Science 84: 364-370 (2010)*). However, specific data on muscle weakness and PPi/ALP levels has not been available. Early data implicated skeletal changes (with a focus on Radiographic Global Impression of Change (RGI-C), but failed to isolate muscle weakness from the phenotypic heterogeneity of HPP.

Notably, the treatment of HPP, particularly the outgoing impairments associated with HPP such as muscle weakness, for an extended period of time, is unknown. Thus, there exists a need for methods that can be used to treat muscle weakness associated with HPP or with other diseases. There additionally exists a need for methods of treatment of hypotonia or muscle weakness in human subjects, as caused by or associated with elevated PPi and/or low alkaline phosphatase activity.

SUMMARY

Muscle weakness has been reported as a symptom in some patients with HPP and in other diseases or disorders. In HPP, elevated PPi concentration is due to loss of function mutation(s) in the gene *ALPL* that encodes the tissue nonspecific isozyme of alkaline phosphatase (TNALP; a.k.a.

liver/bone/kidney type ALP), which is an enzyme for substrates such as inorganic pyrophosphate (PPi), phosphoethanolamine (PEA) and pyridoxal 5'-phosphate (PLP). The instant disclosure teaches methods of treating a muscle weakness disease in a subject characterized with an elevated pyrophosphate (PPi) concentration and/or decreased alkaline phosphatase concentration.

The muscle weakness phenotype of HPP patients may be considered as secondary and caused by the bone mineralization defects, which is taken as the characteristic feature of HPP. Surprisingly, the instant disclosure teaches that muscle weakness in HPP is probably not due to the bone defect, since no difference among the muscles from wild type (WT) mice and *AKP2*$^{-/-}$ mice were observed in their soleus fiber type proportions or soleus or EDL muscle contractile properties *ex vivo*. On the contrary, muscle weakness in HPP was found to be more correlated to the elevated PPi concentration, since reducing PPi by administering asfotase alfa improved *AKP2*$^{-/-}$ mice muscle grip strength. Thus, a subject having a muscle weakness disease characterized by elevated PPi concentration, even without other HPP symptoms or not being diagnosed with HPP yet, may still be treated by asfotase alfa. Methods of testing grip strength have been disclosed, see, e.g., Whyte, M. et al., *Bone* 2016 Dec; 93: 125-138; Whyte, M., et al. *JCI Insight* 2016;27:87-102; Whyte, M. et al., *Bone* 2015 Jun; 75: 229-39.

Disclosed are (1) methods to identify subjects (e.g., humans) having or being prone to a muscle weakness disease for treatment with a recombinant polypeptide having alkaline phosphatase activity (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), and (2) treatment of such subjects with a recombinant polypeptide having alkaline phosphatase activity. Exemplary metrics useful for evaluating the need for or the efficacy of treatment using a recombinant polypeptide having alkaline phosphatase activity include (1) plasma PPi and/or alkaline phosphatase concentration, (2) the Bruininks-Oseretsky Test of Motor Proficiency 2nd Edition (BOT-2), (3) the Childhood Health Assessment Questionnaire (CHAQ), (4) the Pediatric Outcomes Data Collection Instrument (PODCI), (5) Bayley Scales of Infant and Toddler Development, 3rd Edition (BSID-III), (6) the Peabody Developmental Motor Scales, 2nd Edition (PDMS-2), (7) the Six Minute Walk Test (6MWT), (8) the Muscle Strength Grade, and (9) Hand Held Dynamometry (HHD). The methods further include the use of one or more of the described metrics (e.g., plasma PPi concentration, alkaline phosphatase concentration, the BOT-2, the CHAQ, the PODCI, the BSID-III, the PDMS-2, the 6MWT, the Muscle Strength Grade, and HHD) singly or in any combination to assess treatment efficacy using a recombinant polypeptide having alkaline phosphatase activity in a subject having or being prone to a muscle weakness disease in which improvements relative to a certain score or value demonstrate that the recombinant polypeptide having alkaline phosphatase activity is effective for treating a muscle weakness disease.

In one aspect, the instant disclosure provides a method of treating or ameliorating a muscle weakness in a subject having or being prone to a muscle weakness disease, comprising administering to said subject a therapeutically effective amount of at least one recombinant polypeptide having alkaline phosphatase activity. In some embodiments, said subject has an elevated concentration of inorganic pyrophosphate (PPi) and/or low alkaline phosphatase activity or concentration. In one embodiment, said subject has an elevated serum concentration of inorganic pyrophosphate (PPi). In other embodiments,

said subject has an elevated concentration of at least one alkaline phosphatase substrate (e.g., PPi, PLP, PEA, *etc.*).

In another aspect, the instant disclosure also provides a method of identifying a subpopulation of subjects having or being prone to a muscle weakness disease, wherein the subjects in said subpopulation have an elevated inorganic pyrophosphate (PPi) concentration.

In some embodiments, a muscle of said subject is not significantly different from a muscle of a normal subject without said muscle weakness disease in at least one property of such muscle. Such property may be selected from muscle fiber type proportion, fiber contractile properties, or other muscle properties known in the art. Such muscles may include any muscle of the subject, including, e.g., skeletal or striated muscles, cardiac muscles, or smooth muscles. In some embodiments, such muscles include at least one type of arm and leg muscles, particularly at least one type of muscles selected from soleus and *extensor digitorum longus* (EDL) muscles.

In some embodiments, the muscle weakness disease described herein is caused by an elevated concentration of inorganic pyrophosphate (PPi), such as a PPi concentration of greater than about 4.5 μM. In one embodiment, the muscle weakness disease described herein is caused by an elevated serum concentration of inorganic pyrophosphate (PPi). For example, an elevated concentration of PPi in a sample (e.g., a plasma sample) from an infant or child (e.g., a subject less than about 12 years of age) may be about 5.71 μM or greater, an elevated concentration of PPi in a sample (e.g., a plasma sample) from an adolescent (e.g., a subject of about 13 to about 18 years of age) may be about 4.78 μM or greater; and an elevated concentration of PPi in a sample (e.g., a plasma sample) from an adult (e.g., a subject of greater than 18 years of age) may be about 5.82 μM or greater. In other embodiments, the muscle weakness disease described herein is caused by an elevated concentration of at least one alkaline phosphatase substrate (e.g., PPi, PLP, PEA, etc.). In some embodiments, an elevated concentration of pyrophosphate (PPi) enhances the muscle weakness disease described herein in said subject. In one embodiment, an elevated serum concentration of PPi enhances the muscle weakness disease described herein in said subject. For example, an elevated serum concentration of inorganic PPi that enhances the muscle weakness disease can be, e.g, about 5.71 μM or greater in a sample (e.g., a plasma sample) from an infant or child (e.g., a subject less than about 12 years of age), about 4.78 μM or greater in a sample (e.g., a plasma sample) from an adolescent (e.g., a subject of about 13 to about 18 years of age); and about 5.82 μM or greater in a sample (e.g., a plasma sample) from an adult (e.g., a subject of greater than about 18 years of age).

In some embodiments, the muscle weakness disease is caused or enhanced by a low alkaline phosphatase concentration in the subject. For example, the low alkaline phosphatase concentration in a sample (e.g., a plasma sample) from the subject may be, e.g, about 90 U/L or less for a subject of 0 to 14 days of age; about 134 U/L or less for a subject of 15 days of age to less than 1 year of age; about 156 U/L or less for a subject of about 1 year of age to less than 10 years of age; about 141 U/L or less for a subject of about 10 years of age to less than about 13 years of age; about 62 U/L or less for a female subject of about 13 years of age to less than about 15 years of age; about 127 U/L or less for a male subject of about 13 years of age to less than about 15 years of age; about 54 U/L or less for a female subject of about 15 years of age to less than about 17 years of age; about 89 U/L or less for a male

subject of about 15 years of age to less than about 17 years of age; about 48 U/L or less for a female subject of about 17 years of age or older; or about 59 U/L or less for a male subject of about 17 years of age or older.

In other embodiments, an elevated concentration of at least one alkaline phosphatase substrate (e.g., PPi, PLP, PEA, etc.) enhances the muscle weakness disease described herein in said subject.

The muscle weakness disease described herein includes at least one of, e.g., hypophosphatasia (HPP), calcium pyrophosphate dihydrate crystal deposition (CPPD), familial hypophosphatemia (such as autosomal dominant hypophosphatemic rickets (ADHR), autosomal recessive hypophosphatemic rickets, X-linked hypophosphatemic rickets, X-linked hypophosphatemia (XLH), etc.), or other diseases having a muscle weakness phenotype and an elevated concentration of at least one alkaline phosphatase substrate (e.g., PPi, PLP, PEA, etc.). In one embodiment, the muscle weakness disease described herein includes at least one of, e.g., hypophosphatasia (HPP), calcium pyrophosphate dihydrate crystal deposition (CPPD), familial hypophosphatemia (such as autosomal dominant hypophosphatemic rickets (ADHR), autosomal recessive hypophosphatemic rickets, X-linked hypophosphatemic rickets, X-linked hypophosphatemia (XLH), etc.), or other diseases having a muscle weakness phenotype and an elevated concentration (e.g., serum concentration) of inorganic pyrophosphate (PPi).

In some embodiments, administration of at least one recombinant polypeptide having alkaline phosphatase activity (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) reduces the concentration of PPi in a sample (e.g., a plasma sample) from said subject. For example, administration of the at least one recombinant polypeptide having alkaline phosphatase activity to the subject reduces the concentration of PPi in a sample (e.g., a plasma sample) to less than about 5.71 μM for an infant or child (e.g., a plasma PPi concentration of about 3.5 μM, about 4 μM, about 4.5 μM, about 5 μM, or about 5.5 μM or a plasma PPi concentration within the range of about 3.5 μM to about 5.5 μM); less than about 4.78 μM for an adolescent (e.g., a plasma PPi concentration of about 3.5 μM, about 4 μM, or about 4.5 μM, or a plasma PPi concentration within the range of about 3.5 μM to about 4.5 μM); or less than about 5.82 μM for an adult (e.g., a plasma PPi concentration of about 3.5 μM, about 4 μM, about 4.5 μM, about 5 μM, or about 5.5 μM or a plasma PPi concentration within the range of about 3.5 μM to about 5.5 μM).

In another aspect, the instant disclosure also provides a method, comprising: (i) identifying a population of subjects having or being prone to a muscle weakness disease; (ii) identifying a subpopulation of subjects among the population in step (i) wherein: (a) the subjects in said subpopulation have an elevated concentration of inorganic pyrophosphate (PPi); (b) an elevated concentration of inorganic pyrophosphate (PPi) that enhances muscle weakness in the subjects in said subpopulation; or (c) both (a) and (b); and (iii) treating said subpopulation in step (ii).

In another aspect, the instant disclosure also provides a method comprising: (i) identifying a population of subjects having or being prone to a muscle weakness disease; (ii) identifying a subpopulation of subjects among the population in step (i) wherein: (a) the subjects in said subpopulation have an elevated concentration of inorganic pyrophosphate (PPi); (b) an elevated concentration of inorganic pyrophosphate (PPi) that enhances muscle weakness in the subjects in said subpopulation; or

(c) both (a) and (b); and (iii) treating or ameliorating at least one symptom of the muscle weakness disease of a subject in the subpopulation in step (ii), comprising administering to said subject a therapeutically effective amount of at least one recombinant polypeptide having alkaline phosphatase activity. In one embodiment, said subjects in the subpopulation have an elevated serum concentration of inorganic pyrophosphate (PPi).

In some embodiments, the muscle of the subject in step (iii) described herein is not significantly different from the muscle of a normal subject without said type of muscle weakness in at least one property of the muscle. In one embodiment, the at least one property of the muscle includes, e.g., fiber type proportion and/or fiber contractile properties. Such muscles may include any muscle of the subject, including, e.g., skeletal or striated muscles, cardiac muscles, or smooth muscles. In some embodiments, such muscles include at least one type of arm and/or leg muscles, particularly at least one type of muscle selected from soleus and extensor digitorum longus (EDL) muscle. In some embodiments, the method includes identifying a subject having or being prone to a muscle weakness disease and having an elevated concentration of PPi, an elevated concentration of alkaline phosphatase, decreased grip strength, an average BOT-2 strength score of, e.g., less than 10, an average BOT-2 running speed and agility score of, e.g., less than 5, an average CHAQ index score of, e.g., greater than about 0.8, an average PODCI score of, e.g., less than about 40, an average 6MWT of, e.g., less than about 80% of the predicted 6MWT value, and/or a Muscle Strength Grade of, e.g., less than 5.

For example, an elevated concentration of PPi in a sample (e.g., a plasma sample) from an infant or child (e.g., a subject less than about 12 years of age) may be about 5.71 μM or greater, an elevated concentration of PPi in a sample (e.g., a plasma sample) from an adolescent (e.g., a subject of about 13 to about 18 years of age) may be about 4.78 μM or greater; and an elevated concentration of PPi in a sample (e.g., a plasma sample) from an adult (e.g., a subject of greater than about 18 years of age) may be about 5.82 μM or greater. Additionally, an elevated concentration of alkaline phosphatase in a sample (e.g., a plasma sample) from the subject may be, e.g, about 90 U/L or less for a subject of 0 to 14 days of age; about 134 U/L or less for a of 15 days of age to less than 1 year of age; about 156 U/L or less for a subject of about 1 year of age to less than 10 years of age; about 141 U/L or less for a subject of about 10 years of age to less than about 13 years of age; about 62 U/L or less for a female subject of about 13 years of age to less than about 15 years of age; about 127 U/L or less for a male subject of about 13 years of age to less than about 15 years of age; about 54 U/L or less for a female subject of about 15 years of age to less than about 17 years of age; about 89 U/L or less for a male subject of about 15 years of age to less than about 17 years of age; about 48 U/L or less for a female subject of about 17 years of age or older; or about 59 U/L or less for a male subject of about 17 years of age or older.

In some embodiments, the muscle weakness disease described herein is caused by an elevated concentration of inorganic pyrophosphate (PPi). In one embodiment, the muscle weakness disease described herein is caused by an elevated serum concentration of inorganic pyrophosphate (PPi). For example, an elevated concentration of PPi in a sample (e.g., a plasma sample) from an infant or child (e.g., a subject less than about 12 years of age) may be about 5.71 μM or greater, an elevated concentration of PPi in a sample (e.g., a plasma sample) from an adolescent (e.g., a subject of about 13 to about 18 years of age) may be about 4.78 μM or greater; and an elevated concentration of PPi in a

sample (e.g., a plasma sample) from an adult (e.g., a subject of greater than about 18 years of age) may be about 5.82 μM. In other embodiments, the muscle weakness disease described herein is caused by an elevated concentration of at least one alkaline phosphatase substrate (e.g., PPi, PLP, PEA, etc.).

In some embodiments, an elevated concentration of pyrophosphate (PPi) enhances the muscle weakness disease described herein in said subject in step (iii) described herein. In one embodiment, an elevated serum concentration of inorganic pyrophosphate (PPi) enhances the muscle weakness disease described herein in said subject. For example, an elevated concentration of PPi in a sample (e.g., a plasma sample) from an infant or child (e.g., a subject less than about 12 years of age) may be about 5.71 μM or greater, an elevated concentration of PPi in a sample (e.g., a plasma sample) from an adolescent (e.g., a subject of about 13 to about 18 years of age) may be about 4.78 μM or greater; and an elevated concentration of PPi in a sample (e.g., a plasma sample) from an adult (e.g., a subject of greater than about 18 years of age) may be about 5.82 μM

In other embodiments, by an elevated concentration of at least one alkaline phosphatase substrate (e.g., PPi, PLP, PEA, etc.) enhances the muscle weakness disease described herein in said subject.

The muscle weakness disease described herein for subpopulation selection includes at least one of, e.g., hypophosphatasia (HPP), calcium pyrophosphate dihydrate crystal deposition (CPPD), familial hypophosphatemia (such as autosomal dominant hypophosphatemic rickets (ADHR), autosomal recessive hypophosphatemic rickets, X-linked hypophosphatemia (XLH), etc.), or other diseases having a muscle weakness phenotype and an elevated concentration of at least one alkaline phosphatase substrate (e.g., PPi, PLP, PEA, etc.). In one embodiment, the muscle weakness disease described herein includes at least one of, e.g., hypophosphatasia (HPP), calcium pyrophosphate dihydrate crystal deposition (CPPD), familial hypophosphatemia (such as autosomal dominant hypophosphatemic rickets (ADHR), autosomal recessive hypophosphatemic rickets, X-linked hypophosphatemic rickets, X-linked hypophosphatemia (XLH), etc.), or other diseases having a muscle weakness phenotype and an elevated concentration (e.g., serum concentration) of inorganic pyrophosphate (PPi).

In some embodiments, administration of at least one recombinant polypeptide having alkaline phosphatase activity (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) reduces the concentration of inorganic pyrophosphate (PPi) in a sample (e.g., a plasma sample) from said subject. For example, administration of the at least one recombinant polypeptide having alkaline phosphatase activity to the subject reduces the concentration of PPi in a sample (e.g., a plasma sample) to less than about 5.71 μM for an infant or child (e.g., a plasma PPi concentration of about 3.5 μM, about 4 μM, about 4.5 μM, about 5 μM, or about 5.5 μM or a plasma PPi concentration within the range of about 3.5 μM to about 5.5 μM); less than about 4.78 μM for an adolescent (e.g., a plasma PPi concentration of about 3.5 μM, about 4 μM, or about 4.5 μM, or a plasma PPi concentration within the range of about 3.5 μM to about 4.5 μM); or less than about 5.82 μM for an adult (e.g., a plasma PPi concentration of about 3.5 μM, about 4 μM, about 4.5 μM, about 5 μM, or about 5.5 μM or a plasma PPi concentration within the range of about 3.5 μM to about 5.5 μM).

In some embodiments, administration of at least one recombinant polypeptide having alkaline phosphatase activity (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) increases the concentration of alkaline phosphatase in a sample (e.g., a plasma sample) from said subject. For example, administration of the at least one recombinant polypeptide having alkaline phosphatase activity increases the alkaline phosphatase concentration in a sample (e.g., a plasma sample) from the subject to, e.g, about 273 U/L or greater for a subject of 0 to 14 days of age; about 518 U/L or greater for a subject of 15 days of age to less than 1 year of age; about 369 U/L or greater for a subject of about 1 year of age to less than 10 years of age; about 460 U/L or greater for a subject of about 10 years of age to less than about 13 years of age; about 280 U/L or greater for a female subject of about 13 years of age to less than about 15 years of age; about 517 U/L or greater for a male subject of about 13 years of age to less than about 15 years of age; about 128 U/L or greater for a female subject of about 15 years of age to less than about 17 years of age; about 365 U/L or greater for a male subject of about 15 years of age to less than about 17 years of age; about 95 U/L or greater for a female subject of about 17 years of age or older; or about 164 U/L or greater for a male subject of about 17 years of age or older.

In some embodiments, the subject may also exhibit decreased reliance on an assistive mobility device (e.g., a walker, a wheelchair, braces, crutches, and orthotics) after administration of the at least one recombinant polypeptide having alkaline phosphatase activity.

In any of the above aspects, prior to administration of the at least one recombinant polypeptide having alkaline phosphatase activity, the subject is characterized as having an average Hand Held Dynamometry (HHD) value of less than about 80% of a predicted HHD value (e.g., relative to a normal subject of about the same age, the same gender, and/or the same height), in particular, in which the HHD value represents the grip strength, knee flexion, knee extension, hip flexion, hip extension, or hip abduction of the subject. For example, administration of the at least one recombinant polypeptide having alkaline phosphatase activity results in an average HHD value of the subject of about 50% or more of a predicted HHD value, e.g., in which the HHD value represents the grip strength, knee flexion, knee extension, hip flexion, hip extension, or hip abduction of the subject..

In some embodiments, the at least one recombinant polypeptide having alkaline phosphatase activity described herein is or can be administered to the subject daily, twice a week, once a week, or in even lower frequency. In one embodiment, the at least one recombinant polypeptide having alkaline phosphatase activity described herein is administered to the subject daily. The at least one recombinant polypeptide having alkaline phosphatase activity described herein can be administered to the subject for at least one week, two weeks, one month, three months, six months, one year, or a longer period, up to the whole life of the subject.

In some embodiments, the at least one recombinant polypeptide having alkaline phosphatase activity described herein is or can be administered by at least one route. Such routes include, e.g., subcutaneous, intravenous, intramuscular, sublingual, intrathecal, intradermal, or other routes known in the art. In one embodiment, the at least one recombinant polypeptide having alkaline phosphatase activity described herein is administered subcutaneously.

In some embodiments, the at least one recombinant polypeptide having alkaline phosphatase activity described herein comprises at least one of a tissue nonspecific alkaline phosphatase (TNALP), a placental alkaline phosphatase (PALP), a germ cell alkaline phosphatase (GCALP), an intestinal alkaline phosphatase (IALP), and biologically functional fragments, fusions, or chimeric constructs thereof. In one embodiment, the at least one recombinant polypeptide having alkaline phosphatase activity described herein comprises at least one of a soluble fragment of TNALP, PALP, GCALP, and IALP. In one embodiment, the tissue nonspecific alkaline phosphatase (TNALP) described herein comprises or consists of an amino acid sequence of the amino acids 1-485 of SEQ ID NO: 1. In another embodiment, the tissue nonspecific alkaline phosphatase (TNALP) described herein comprises or consists of an amino acid sequence of SEQ ID NO: 1.

In some embodiments, the at least one recombinant polypeptide having alkaline phosphatase activity described herein is a fusion protein. In one embodiment, the at least one recombinant polypeptide having alkaline phosphatase activity described herein comprise an immunoglobulin molecule. Such immunoglobulin molecule may be, e.g., a fragment crystallizable region (Fc), or a full-length, or fragment thereof of, an IgG, including but not limited to IgG_1, IgG_2, IgG_3, IgG_4, $IgG_{2/4}$, or other IgG fusions. In one embodiment, the Fc described herein comprises an amino acid sequence of SEQ ID NO: 20.

In some embodiments, the at least one recombinant polypeptide having alkaline phosphatase activity described herein comprises a negatively charged peptide. Such negatively charged peptide may include at least one poly(glutamic acid) (polyE) or a poly(aspartic acid) (polyD) peptide, e.g., the at least one recombinant polypeptide having alkaline phosphatase activity includes 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 consecutive acidic residues, in particular, aspartic acid (D) or glutamic acid (E), such as at least one of D_{10}, D_{16}, E_{10}, and E_{16}. In some embodiments, the at least one recombinant polypeptide having alkaline phosphatase activity includes E_6, E_7, E_8, E_9, E_{10}, E_{11}, E_{12}, E_{13}, E_{14}, E_{15}, E_{16}, D_6, D_7, D_8, D_9, D_{10}, D_{11}, D_{12}, D_{13}, D_{14}, D_{15}, or D_{16}, e.g., E_6, E_{10}, D_6, or D_{10}.

In some embodiments, the at least one recombinant polypeptide having alkaline phosphatase activity described herein comprises a bone targeted alkaline phosphatase comprising a polypeptide having the structure: Z-sALP-Y-spacer-X-Wn-V, wherein sALP is the extracellular domain of the alkaline phosphatase; V is absent or is an amino acid sequence of at least one amino acid; X is absent or is an amino acid sequence of at least one amino acid; Y is absent or is an amino acid sequence of at least one amino acid; Z is absent or is an amino acid sequence of at least one amino acid; and Wn is a polyaspartate or a polyglutamate wherein n=10 to 16.

In some embodiments, the spacer described herein comprises a fragment crystallizable region (Fc). In one embodiment, the Fc described herein comprises an amino acid sequence of SEQ ID NO: 20.

In some embodiments, the at least one recombinant polypeptide having alkaline phosphatase activity described herein comprises a structure of sALP-Fc-D_{10}.

In one embodiment, the at least one recombinant polypeptide having alkaline phosphatase activity described herein comprises a dimer comprising monomers of an amino acid sequence of SEQ ID NO: 1.

In some embodiments, the at least one recombinant polypeptide having alkaline phosphatase activity described herein is administered in a dosage from about 0.1 mg/kg/day to about 20 mg/kg/day, or a comparable weekly dosage. In one embodiment, the at least one recombinant polypeptide having

alkaline phosphatase activity described herein is administered in a dosage from about 0.5 mg/kg/day to about 20 mg/kg/day, or a comparable weekly dosage. In another embodiment, the at least one recombinant polypeptide having alkaline phosphatase activity described herein is administered in a dosage from about 0.5 mg/kg/day to about 10 mg/kg/day, or a comparable weekly dosage. In another embodiment, the at least one recombinant polypeptide having alkaline phosphatase activity described herein is administered in a dosage from about 1 mg/kg/day to about 10 mg/kg/day, or a comparable weekly dosage.

In some embodiments, the subject described herein is a mammal (e.g., a human).

Definitions

As used herein, "a" or "an" means "at least one" or "one or more" unless otherwise indicated. In addition, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise.

As used herein, "about" refers to an amount that is ± 10 % of the recited value and is preferably ± 5 % of the recited value, or more preferably ± 2 % of the recited value.

As used herein, "at least" refers to an amount that is ≤ 10 % of the recited value and is preferably ≤ 5 % of the recited value, or more preferably ≤ 2 % of the recited value.

By "asfotase alfa" is meant a human TNALP (hTNALP) fusion protein formulated for the treatment of HPP. Asfotase alfa is a fusion protein including a soluble glycoprotein of two identical polypeptide chains, in which each polypeptide chain includes amino acid residues 1-726 of SEQ ID NO: 1. The structure of each polypeptide chain includes the catalytic domain of hTNALP, the human immunoglobulin G_1 Fc domain, and a deca-aspartate peptide used as a bone targeting domain (the structure hTNALP-Fc-D_{10}). The two polypeptide chains are covalently linked by two disulfide bonds. Asfotase alfa has been approved under the trade name STRENSIQ® (Alexion Pharmaceuticals, Inc., New Haven, CT) in the United States, Europe, Japan, Canada, Israel, Australia, and Korea.

The terms "individual," "subject" and "patient" are used interchangeably and refer to any subject for whom diagnosis, treatment or therapy is desired, particularly humans. Other subjects may include, for example, cattle, dogs, cats, guinea pigs, rabbits, rats, mice, horses and the like. As used herein, an "at risk" subject or a subject "being prone to" a disease is a subject who is identified as having a risk of developing a disease, disorder or symptoms associated with a muscle weakness disease.

As used herein, "average" refers to a numerical value expressing the mean or median of a data set. The mean of a data set is calculated by dividing the sum of the values in the data set by their number. The median of a data set is calculated by determining the middle value in a list data of odd numbers or by determining the mean of the two data values in the middle in a list of even numbers.

The term "wild-type" "or "wild-type sequence" used for TNALP or other genes or proteins in the instant disclosure refers to the typical form of such genes or proteins as it occurs in nature in normal human, non-human mammals, or other living organisms. A wild-type sequence may refer to the standard "normal" allele at a locus for a gene or the standard "normal" primary amino acid sequence (optionally with the standard "normal" post-translational modifications to and/or inter-chain bonds and/or interactions among amino acid residues) for a polypeptide or protein, in contrast to that produced by a non-standard,

"mutant" allele or amino acid sequence/modification/interaction. "Mutant" alleles can vary to a great extent, and even become the wild type if a genetic shift occurs within the population. It is now appreciated that most or all gene loci (and less frequently, but still possible, for most polypeptide sequences) exist in a variety of allelic forms, which vary in frequency throughout the geographic range of a species, and that a uniform wild type may not necessarily exist. In general, however, the most prevalent allele or amino acid sequence – i.e., the one with the highest frequency among normal individual human or other organisms - is the one deemed as wild type in the instant disclosure.

The term "normal" used for human or other organisms in this specification refers to, except for specified otherwise, a human or other organisms without any diseases (e.g., HPP), disorders, and/or symptoms or physiological consequences (e.g., muscle weakness) caused by or related to the aberrant activity (which may be due to, e.g., deficient or lack of gene or protein product and/or defective or loss-of-function of gene or protein product) of the relevant gene or polypeptide/protein. The most obvious example for a normal human is a human being who lacks muscle weakness or muscle weakness symptoms and lacks mutations or modifications to genes or proteins (e.g., the ALPL gene and ALP proteins) which may result in HPP-related muscle weakness. In another scenario focusing on ALP functions, the scope of a "normal" human in the present disclosure may be broadened to include any human beings having no aberrant endogenous alkaline phosphatase activity (which may be tested by, e.g., the substrate (PPi, PEA and PLP) levels and compared to the corresponding activity in other healthy or normal human beings).

As used herein, an "elevated" or "increased" concentration refers to a concentration (e.g., of PPi) in a subject having or being prone to a muscle weakness disease described herein which is higher than the concentration in a wild-type subject, in another subject without the muscle weakness disease, in the same subject at a time point when the subject has no such muscle weakness disease, or in the same subject should the subject have had no such muscle weakness disease. Such "elevated concentration" refers to an elevated concentration inside the subject described herein, including any cell, tissue, organ, or part of the subject. In one embodiment, such "elevated concentration" comprises an elevated concentration in the serum of the subject.

The terms "Bayley Scales of Infant and Toddler Development, 3rd Edition" or "BSID-III" as used herein refer to a standardized series of measurements used to assess the motor (fine and gross), language (receptive and expressive), and cognitive development of patients. See Bayley, (2006). *Bayley scales of infant and toddler development: administration manual*. San Antonio, TX: Harcourt Assessment, hereby incorporated by reference in its entirety. The BSID-III measurements include a series of developmental play tasks to be administered to the patient. Raw scores of successfully completed items are converted to scaled scores. The scaled scores are then used to determine the patient's performance compared to healthy, age-adjusted patients. The BSID-III can also include the Social-Emotional Adaptive Behavior Questionnaire, which is completed by the parent/guardian, to establish the range of adaptive behaviors of the patient. For example, measurements for determining the BSID-III score (e.g., the BSID-III gross motor function score) can include prehension, perceptual-motor integration, motor planning and speed, visual tracking, reaching, object grasping, object manipulation, functional hand skills, responses to tactile information, movement of the limbs and torso, static positioning, dynamic movement, balance, and

motor planning. These patient measurements are then converted into a BSID-III scaled score (e.g., the BSID-III gross motor function scaled score) ranging from 0 to 14, in which scores of about 7 to about 13 are considered the normal range of healthy patients.

The term "bone-targeting moiety," as used herein, refers to an amino acid sequence of between 1 and 50 amino acid residues in length having a sufficient affinity to the bone matrix, such that the bone-targeting moiety, singularly, has an *in vivo* binding affinity to the bone matrix of about 10^{-6} M to about 10^{-15} M (e.g., 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, 10^{-13} M, 10^{-14} M, or 10^{-15} M).

The terms "Bruininks-Oseretsky Test of Motor Proficiency 2nd Edition" or "BOT-2," as used herein, refer to the second edition of a standardized test of gross and fine motor performance for patients, e.g., from about 4 to about 21 years of age. See Bruininks, R. H. (2005). *Bruininks-Oseretsky Test of Motor Proficiency, (BOT-2)*. Minneapolis, MN: Pearson Assessment, hereby incorporated by reference in its entirety. The BOT-2 is administered individually to assess gross and fine motor skills of a range of patients. In particular, the BOT-2 can be used to evaluate physical impairments and mobility restrictions in patients having HPP. The BOT-2 provides composite BOT-2 scores in the following areas: strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and upper-limb coordination. For example, a BOT-2 strength score can be determined by having a patient perform sit-ups, v-ups, standing long jump, wall sit, and push-ups. A running speed and agility score can be determined by having a patient step over a balance beam or perform a shuttle run, two-legged side hop, or one-legged side hop. Both BOT-2 strength and BOT-2 running speed and agility scores range from 0 to 25, in which a score of about 10 to 20 is considered representative of healthy patients.

The terms "Childhood Health Assessment Questionnaire" or "CHAQ," as used herein refer to a questionnaire that is used to assess the health status (e.g., ability to perform activities of daily living (ADLs) and incidence of pain) of patients of 1 to 19 years of age, such as patients with HPP. For a description of the CHAQ index, see Bruce & Fries (*J. Rheumatol.* 30(1): 167-178, 2003), hereby incorporated by reference in its entirety. The CHAQ may be administered by interview or self-report for children greater than 8 years of age. The CHAQ includes eight sub-scales for dressing/grooming, arising, eating, walking, hygiene, reach, grip, and activities. The range of scores within each category is from 0 to 3, in which a score of 0 indicates without any difficulty; a score of 1 indicates with some difficulty; a score of 2 indicates with much difficulty; and a score of 3 indicates that the patient is unable to perform the activity. The CHAQ index may also be used to determine the presence and severity of pain.

By "extracellular domain" is meant any functional extracellular portion of the native protein, e.g., alkaline phosphatase. In particular, the extracellular domain lacks the signal peptide.

By "Fc" is meant a fragment crystallizable region of an immunoglobulin, e.g., IgG-1, IgG-2, IgG-3, IgG-3 or IgG-4, including the CH2 and CH3 domains of the immunoglobulin heavy chain. Fc may also include any portion of the hinge region joining the Fab and Fc regions. The Fc can be of any mammal, including human, and may be post-translationally modified (e.g., by glycosylation). In a non-limiting example, Fc can be the fragment crystallizable region of human IgG-1 having the amino acid sequence of SEQ ID NO: 20.

By "fragment" is meant a portion of a polypeptide or nucleic acid molecule that contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain, e.g., 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 400, 500, 600, 700, or more amino acid residues, up to the entire length of the polypeptide. Exemplary sALP fragments have amino acid residues 18-498, 18-499, 18-500, 18-501, 18-502, 18-503, 18-504, 18-505, 18-506, 18-507, 18-508, 18-509, 18-510, 18-511, or 18-512 of a ALP (e.g., SEQ ID NOs: 2-6), and may include additional C-terminal and/or N-terminal portions.

The terms "Hand Held Dynamometry" and "HHD" as used interchangeably herein refer to a method to measure the grip and muscle strength of subjects, in particular, subjects having or being prone to a muscle weakness disease. A dynamometer can be used to assess grip strength, knee flexion, knee extension, hip flexion, hip extension, and hip abduction of a subject (e.g., a subject having or being prone to a muscle weakness disease). For example, knee flexion and extension and also hip flexion, extension, and abduction of a subject having or being prone to a muscle weakness disease can be measured using, e.g., a MICROFET2™ Dynamometer, while grip strength of the subject can be measured using, e.g., a Jamar Grip Dynamometer. In particular, the administrator holds the dynamometer stationary, and the subject exerts a maximal force against the dynamometer. Peak force data is collected in pounds, then converted to Newtons (N). Torque values are then calculated using limb length in N-meters. The torque value can then be compared to the value of, e.g., a normal subject of about the same age, the same gender, and/or the same height, and expressed as a percentage value to generate the HHD value of the subject.

The terms "hypophosphatasia" or "HPP," as used herein, refer to a rare, heritable skeletal disorder caused by, e.g., one or more loss-of-function mutations in the ALPL (alkaline phosphatase, liver/bone/kidney) gene, which encodes tissue-nonspecific alkaline phosphatase (TNALP). HPP may be further characterized as infantile HPP, childhood HPP, perinatal HPP (e.g., benign perinatal HPP or lethal perinatal HPP), or odonto-HPP.

By "naïve patient" or "naïve subject" is meant a patient or subject having a muscle weakness disease described herein that has never received treatment with an alkaline phosphatase, or a polypeptide having alkaline phosphatase activity, such as a sALP (e.g., TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa).

By "pain" as used herein refers to physical suffering or discomfort caused by a muscle weakness disease described herein, such as muscle pain. For instance, symptoms of pain can include, e.g., soreness, tightness, or stiffness. The severity of pain can vary between patients (e.g., chronic pain or acute pain). In particular, chronic pain refers to pain that lasts longer than three to six months or pain that extend beyond the expected period of healing. In contrast, acute pain refers to pain that typically lasts less than three to six months. As described herein, therapeutic compositions (e.g., including a sALP, such as asfotase alfa) can be administered to a patient suffering from pain (e.g., muscle pain) in an amount sufficient to relieve or at least partially relieve the symptoms of pain (e.g., discomfort, soreness,

tightness, or stiffness) and its complications (e.g., fatigue, sleeplessness, weakened immune system, depression, anxiety, stress, irritability, or disability).

The terms "peptide," "polypeptide," and "protein" are used interchangeably and refer to any chain of two or more natural or unnatural amino acid residues, regardless of post-translational modification (e.g., glycosylation or phosphorylation), constituting all or part of a naturally-occurring or non-naturally occurring polypeptide or peptide, as is described herein.

By "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient" is meant at least one carrier or excipient, respectively, which is physiologically acceptable to the treated patient while retaining the therapeutic properties of the compound with which it is administered. One exemplary pharmaceutically acceptable carrier substance is physiological saline. For instance, the pharmaceutically acceptable carrier can include sodium chloride (e.g., 150 mM sodium chloride) and sodium phosphate (e.g., 25 mM sodium phosphate). Other physiologically acceptable carriers and their formulations are known to those skilled in the art and described, e.g., in Remington's Pharmaceutical Sciences (20th edition), A. Gennaro, Ed., 2000, Lippincott, Williams & Wilkins, Philadelphia, PA.

By "pharmaceutical composition" is meant a composition containing a polypeptide or nucleic acid molecule as described herein formulated with at least one pharmaceutically acceptable excipient, diluent, or carrier. The pharmaceutical composition may be manufactured or sold with the approval of a governmental regulatory agency as part of a therapeutic regimen for the treatment or prevention of a disease or event in a patient. Pharmaceutical compositions can be formulated, for example, for subcutaneous administration, intravenous administration (e.g., as a sterile solution free of particulate emboli and in a solvent system suitable for intravenous use), for oral administration (e.g., a tablet, capsule, caplet, gelcap, or syrup), or any other formulation described herein, e.g., in unit dosage form. In one embodiment, the pharmaceutical composition of the present disclosure is subcutaneously administered or is formulated for subcutaneous administration.

The term "physical impairments," as used herein, refers to a physiological condition, such as bone weakness and muscle weakness diseases described herein that can restrict or eliminate, e.g., ambulation, functional endurance, and ability to perform activities of daily living (ADL) of a patient. In particular, physical impairments may restrict or eliminate a patient's ability to perform ADL, which are routine activities that healthy patients perform on a daily basis without requiring assistance, such as functional mobility or transferring (e.g., walking), bathing and showering, dressing, self-feeding, and personal hygiene and grooming. As described herein, therapeutic compositions (e.g., compositions including a sALP, such as asfotase alfa) can be administered to a patient to decrease the severity and/or frequency of physical impairments associated with muscle weakness.

The terms "Pediatric Outcomes Data Collection Instrument" or "PODCI," as used herein, refer to a questionnaire used to assess overall health, incidence of pain, and ability to perform ADLs of patients under 19 years of age, particularly in patients with chronic health disorders, such as patients with HPP. For a description of the PODCI, see Plint et al. (*J. Pediatr. Orthop. 23*(6): 788-790, 2003), hereby incorporated by reference in its entirety. The questionnaire may be completed by the patient or by a parent/guardian of the patient with knowledge of the patient's condition. The eight scales generated from the PODCI include the following: 1) the upper extremity and physical function scale to measure difficulty

encountered in performing daily personal care and student activities; 2) the transfer and basic mobility scale to measure difficulty experienced in performing routine motion and motor activities in daily activities; 3) the sports/physical functioning scale to measure difficulty or limitations encountered in participating in more active activities or sports; 4) the pain/comfort scale to measure the level of pain experienced during the past week; 5) the treatment expectations scale to measure the long term expectations of treatment; 6) the happiness scale to measure overall satisfaction with personal looks and sense of similarity to friends and others of own age; 7) the satisfaction with symptoms scale to measure the patient's acceptance of current limitations should this be a life-long state; and 8) the global functioning scale, which is a general combined scale calculated from the first four scales listed above. Standardized scores are generated from a series of questions in the PODCI and converted to a 0 to 100 scale, in which 0 represents significant disability and 100 represents less disability.

The terms "Peabody Developmental Motor Scales, 2nd Edition" or "PDMS-2," as used herein, refer to an early childhood motor development program that provides an assessment of gross and fine motor skills in patients from birth throughout childhood (e.g., infants and children). For a description of the PDMS-2 scales, see van Hartingsveldt et al. (*Occup. Ther. Int.* 12(1): 1-13, 2005), hereby incorporated by reference in its entirety. The PDMS-2 is composed of six subtests that measure interrelated motor abilities of early development. The six subtests include the following: 1) the locomotor subtest to measures a patient's ability to move from one place to another (measurements include crawling, walking, running, hopping, and jumping forward); 2) the reflexes subtest to measure a patient's ability to automatically react to environmental events; 3) the stationary subtest to measure a patient's ability to sustain control of his or her body within the center of gravity and retain equilibrium; 4) the object manipulation subtest to measure a patient's ability to manipulate an object, such as catching, throwing, and kicking a ball; 5) the grasping subtest to measure a patient's ability to use his or her hands, such as the ability to hold an object with one hand and actions involving the controlled use of the fingers of both hands; and 6) the visual-motor integration subtest to measure a patient's ability to use his or her visual perceptual skills to perform complex eye-hand coordination tasks, such as reaching and grasping for an object, building with blocks, and copying designs. The PDMS-2 measurements for each subtest is converted into a PDMS-2 score, such as the PDMS-2 locomotor standard score ranging from 0 to 13, in which the range of health patients is from about 7 to about 13.

The terms "sALP," "soluble alkaline phosphatase," and "extracellular domain of an alkaline phosphatase" are used interchangeably and refer to a soluble, non-membrane-bound alkaline phosphatase or a domain, biologically active fragment, or biologically active variant thereof. sALPs include, for example, an alkaline phosphatase lacking a C-terminal glycolipid anchor (GPI signal sequence, e.g., polypeptides including or consisting of the amino acid residues 18-502 of a human TNALP (SEQ ID NOs: 2, 3, 4, 5, or 6)). In particular, a TNALP may include, e.g., a polypeptide including or consisting of amino acid residues 1-485 of SEQ ID NO: 1, such as asfotase alfa, or a polypeptide variant having at least 95% sequence identity to the amino acid residues 1-485 of SEQ ID NO: 1. sALPs further include, for example, mammalian orthologs of human TNALP, such as a rhesus TNALP (SEQ ID NO: 7), a rat TNALP (SEQ ID NO: 8), a canine TNALP (SEQ ID NO: 9), a porcine TNALP (SEQ ID NO: 10), a murine TNALP (SEQ ID NO: 11), a bovine TNALP (SEQ ID NOs: 12-14), or a feline TNALP (SEQ

ID NO: 15). sALPs also include soluble, non-membrane-bound forms of human PALP (e.g., polypeptides including or consisting of amino acid residues 18-502 of SEQ ID NOs: 16 or 17), GCALP (e.g., polypeptides including or consisting of amino acid residues 18-502 of SEQ ID NO: 18), and IALP (e.g., polypeptides including or consisting of amino acid residues 18-502 of SEQ ID NO: 19), and additional variants and analogs thereof that retain alkaline phosphatase activity, e.g., the ability to hydrolyze PPi. A sALP, in particular, lacks the N-terminal signal peptide (e.g., aa 1-17 of SEQ ID NOs: 2-6, 8, 11-13, or 15 or aa 1-25 of SEQ ID NO: 7).

By "sALP polypeptide" is meant a polypeptide having the structure A-sALP-B, wherein sALP is as defined herein and each of A and B is absent or is an amino acid sequence of at least one amino acid. An exemplary sALP polypeptide has an amino acid sequence comprising or consisting of the amino acids 1-485 of SEQ ID NO: 1. Other exemplary sALP polypeptides include any sALP fusion polypeptides described herein (for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa).

By "signal peptide" is meant a short peptide (5-30 amino acids long) at the N-terminus of a polypeptide that directs a polypeptide towards the secretory pathway (e.g., the extracellular space). The signal peptide is typically cleaved during secretion of the polypeptide. The signal sequence may direct the polypeptide to an intracellular compartment or organelle, e.g., the Golgi apparatus. A signal sequence may be identified by homology, or biological activity, to a peptide with the known function of targeting a polypeptide to a particular region of the cell. One of ordinary skill in the art can identify a signal peptide by using readily available software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, or PILEUP/PRETTYBOX programs). A signal peptide can be one that is, for example, substantially identical to amino acid residues 1-17 of SEQ ID NOs: 2-6 or amino acid residues 1-25 of SEQ ID NO: 7.

As used herein, when a polypeptide or nucleic acid sequence is referred to as having "at least X% sequence identity" to a reference sequence, wherein "X" is a real number, it is meant that at least X percent of the amino acid residues or nucleotides in the polypeptide or nucleic acid are identical to those of the reference sequence when the sequences are optimally aligned. An optimal alignment of sequences can be determined in various ways that are within the skill in the art, for instance, the Smith Waterman alignment algorithm (Smith et al., *J. Mol. Biol.* 147:195-7, 1981) and BLAST (Basic Local Alignment Search Tool; Altschul et al., *J. Mol. Biol.* 215: 403-10, 1990). These and other alignment algorithms are accessible using publicly available computer software such as "Best Fit" (Smith and Waterman, Advances in Applied Mathematics, 482-489, 1981) as incorporated into GeneMatcher Plus (Schwarz and Dayhoff, Atlas of Protein Sequence and Structure, Dayhoff, M.O., Ed pp 353-358, 1979), BLAST, BLAST-2, BLAST-P, BLAST-N, BLAST-X, WU-BLAST-2, ALIGN, ALIGN-2, CLUSTAL, Megalign (DNASTAR), or other software/hardware for alignment. In addition, those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve optimal alignment over the length of the sequences being compared.

The terms "patient" and "subject" are used interchangeably and refer to a mammal, including, but not limited to, a human or a non-human mammal, such as a bovine, equine, canine, ovine, or feline.

By "therapeutically effective amount" is meant an amount of a polypeptide or nucleic acid molecule described herein that is sufficient to substantially improve, treat, prevent, delay, suppress, or arrest at least one symptom of HPP. A therapeutically effective amount of a composition described herein may depend on the severity of the disorder being treated and the condition, weight, and general state of the patient and can be determined by an ordinarily-skilled artisan with consideration of such factors. A therapeutically effective amount of a composition described herein can be administered to a patient in a single dose or in multiple doses administered over a period of time.

By "treating," "treat," or "treatment" is meant the medical management of a patient with the intent to cure, ameliorate, stabilize, reduce the likelihood of, or prevent a muscle weakness diseases (e.g., in a patient with HPP) and/or management of a patient exhibiting or likely to have a muscle weakness diseases (e.g., in a patient with HPP), e.g., by administering a pharmaceutical composition.

This term includes active treatment, that is, treatment directed specifically toward the improvement or associated with the cure of a disease, pathological condition, disorder, or event, and also includes causal treatment, that is, treatment directed toward removal of the cause of the associated disease, pathological condition, disorder, or event. In addition, this term includes palliative treatment, that is, treatment designed for the relief or improvement of at least one symptom rather than the curing of the disease, pathological condition, disorder, or event; symptomatic treatment, that is, treatment directed toward constitutional symptoms of the associated disease, pathological condition, disorder, or event; preventative treatment, that is, treatment directed to minimizing or partially or completely inhibiting the development of the associated disease, pathological condition, disorder, or event, e.g., in a patient who is not yet ill, but who is susceptible to, or otherwise at risk of, a particular disease, pathological condition, disorder, or event; and supportive treatment, that is, treatment employed to supplement another specific therapy directed toward the improvement of the associated disease, pathological condition, disorder, or event.

As used herein, "walking ability" refers to the ability of a patient (e.g., a patient having a muscle weakness disease described herein) to lift and set down each foot in turn. Walking ability may be assessed by tests, in particular, the Six-Minute Walk Test (6MWT). See the American Thoracic Society statement: guidelines for the six-minute walk test (*American Journal of Respiratory and Critical Care Medicine*, 166(1):111-7, 2002), hereby incorporated by reference in its entirety.

Other features and advantages of the present disclosure will be apparent from the following Detailed Description, the drawings, and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph showing the percentage of myosin fiber type in all soleus muscle fibers from wild type (WT) mice or *Akp2*$^{-/-}$ mice.

FIGS. 2A-2D are graphs of fiber size distribution in the soleus muscles dissected from wild type (WT) mice or *Akp2*$^{-/-}$ mice. The percentages of fibers of different minimum sizes (μm^2) are shown for all fibers (Fig. 2A), type I fibers (Fig. 2B), type IIa fibers (Fig. 2C), or type IIb fibers (Fig. 2D).

FIGS. 3A-3D are graphs of contractile properties of the soleus muscles from wild type (WT) mice or *Akp2*$^{-/-}$ mice. Muscle mass (Fig. 3A), strength (Fig. 3B), force frequency (Fig. 3C), and fatigue

characteristics (Fig. 3D) were compared in both male and female mice.

FIGS. 4A-4D are graphs of contractile properties of the extensor digitorum longus (EDL) muscles from wild type (WT) mice or *Akp2*$^{-/-}$ mice. Muscle mass (Fig. 4A), strength (Fig. 4B), force frequency (Fig. 4C), and fatigue characteristics (Fig. 4D) were compared in both male and female mice.

FIGS. 5A-5B are graphs of contractile properties of the dissected soleus (Fig. 5A) and extensor digitorum longus (EDL) (Fig. 5B) muscles from wild type (WT) mice or *Akp2*$^{-/-}$ mice in related to PPi concentration.

FIG. 6 is a graph of grip strength of forelimbs or hindlimbs of wild type (WT) mice, *Akp2*$^{-/-}$ mice receiving continuous treatment of asfotase alfa (Tx-Tx) after Day 35, or *Akp2*$^{-/-}$ mice with discontinued treatment of asfotase alfa (Tx-V) after Day 35.

DETAILED DESCRIPTION

Muscle weakness has been reported as one of the several symptoms of HPP (Seshia et al. 1990 *Archives of Disease in Childhood* 65:130-131). In addition to HPP, other diseases or disorders may also lead to muscle weakness. For example, magnesium shortage results in muscle weakness in calcium pyrophosphate deposition disease (CPPD, or CPDD) patients (Hahn et al. 2012 *BMC Gastroenterology* 12-19). Some muscle weakness diseases or disorders, such as HPP, CPPD, familial hypophosphatemia (such as autosomal dominant hypophosphatemic rickets (ADHR), autosomal recessive hypophosphatemic rickets, X-linked hypophosphatemic rickets, X-linked hypophosphatemia (XLH), etc.), share a characteristic feature of elevated pyrophosphate (PPi) concentration in the subject suffering the diseases or disorders. In HPP, the elevated PPi concentration is due to the loss of function mutation(s) in the gene *ALPL* that encodes the tissue nonspecific isozyme of alkaline phosphatase (TNALP; a.k.a. liver/bone/kidney type ALP), which is an enzyme for substrates such as PPi, phosphoethanolamine (PEA) and pyridoxal 5'-phosphate (PLP). In CPPD, a deficiency of Mg, which acts as a cofactor for various phosphatases, leads to higher amounts of PPi, which is a necessary precursor for the formation of CPPD crystals. The deposition of calcium pyrophosphate may further lead to chronic inflammatory arthritis, hypophosphatasia, hypomagnesemia, and hyperparathyroidism with chondrocalcinosis and acute attacks of "pseudogout."

The instant disclosure teaches methods of treating a muscle weakness disease in a subject characterized as having one or more of the following: an elevated PPi concentration, decreased alkaline phosphatase concentration, an average BOT-2 strength score of, e.g., less than 10, an average BOT-2 running speed and agility score of, e.g., less than 5, an average CHAQ index score of, e.g., greater than about 0.8, or an average PODCI score of, e.g., less than about 40, an average 6MWT of, e.g., less than about 80% of the predicted 6MWT value (e.g., in which the predicted 6MWT value is the 6MWT value of an age-matched and/or gender-matched normal subject), a Muscle Strength Grade of, e.g., less than 5, and/or an average HHD value (e.g., an average HHD muscle or grip strength value) of, e.g., less than about 50% of the predicted HHD value (e.g., in which the predicted HHD value is the HHD value of an age-matched and/or gender-matched normal subject). In particular, the subject has been identified as having or being prone to a muscle weakness.

For example, disclosed are methods of identifying subjects (e.g., humans) having or being prone to a muscle weakness disease for treatment with a recombinant polypeptide having alkaline phosphatase activity (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) characterized as having an elevated PPi concentration, decreased ALP concentration, an average BOT-2 strength score of, e.g., less than 10, an average BOT-2 running speed and agility score of, e.g., less than 5, an average CHAQ index score of, e.g., greater than about 0.8, an average PODCI score of, e.g., less than about 40, an average 6MWT of, e.g., less than about 80% of the predicted 6MWT value, a Muscle Strength Grade of, e.g., less than 5, and/or an average HHD value (e.g., an average HHD muscle or grip strength value) of, e.g., less than about 80% of the predicted HHD value. For example, an elevated concentration of PPi in a sample (e.g., a plasma sample) from an infant or child (e.g., a subject less than about 12 years of age) may be about 5.71 μM or greater, an elevated concentration of PPi in a sample (e.g., a plasma sample) from an adolescent (e.g., a subject of about 13 to about 18 years of age) may be about 4.78 μM or greater; and an elevated concentration of PPi in a sample (e.g., a plasma sample) from an adult (e.g., a subject of greater than about 18 years of age) may be about 5.82 μM or greater. In particular, a decreased ALP concentration in a sample (e.g., a plasma sample) from the subject may be, e.g, about 90 U/L or less for a subject of 0 to 14 days of age; about 134 U/L or less for a subject of 15 days of age to less than 1 year of age; about 156 U/L or less for a subject of about 1 year of age to less than 10 years of age; about 141 U/L or less for a subject of about 10 years of age to less than about 13 years of age; about 62 U/L or less for a female subject of about 13 years of age to less than about 15 years of age; about 127 U/L or less for a male subject of about 13 years of age to less than about 15 years of age; about 54 U/L or less for a female subject of about 15 years of age to less than about 17 years of age; about 89 U/L or less for a male subject of about 15 years of age to less than about 17 years of age; about 48 U/L or less for a female subject of about 17 years of age or older; or about 59 U/L or less for a male subject of about 17 years of age or older.

The instant disclosure provides a method of treating or ameliorating a muscle weakness in a subject having or being prone to a muscle weakness disease, comprising administering to said subject a therapeutically effective amount of at least one recombinant polypeptide having alkaline phosphatase activity. In particular, the subject has been identified as having or being prone to a muscle weakness.

The instant disclosure also provides a method of identifying a subpopulation of subjects having or being prone to a muscle weakness disease, wherein the subjects in said subpopulation have elevated PPi concentrations, decreased alkaline phosphatase concentrations, and/or decreased grip or muscle strength (e.g., as assessed using the BOT-2, 6MWT, CHAQ, PODCI, Muscle Strength Grade, and/or HHD).

Methods for: 1) identifying a subpopulation of subjects having or being prone to a muscle weakness disease, wherein the subjects in said subpopulation have elevated PPi concentrations, decreased ALP concentrations, and/or decreased grip or muscle strength; and 2) then treating or ameliorating at least one symptom of the muscle weakness disease in a subject in said subpopulation are also described.

Methods of identifying a subpopulation of subjects having or being prone to a muscle weakness disease characterized with elevated PPi concentrations, decreased ALP concentrations, and/or decreased grip or muscle strength are also described.

The subpopulation of subjects can be identified irrespective of whether they have previously been diagnosed with hypophosphatasia (HPP), calcium pyrophosphate deposition disease (CPPD), or familial hypophosphatemia (such as autosomal dominant hypophosphatemic rickets (ADHR), autosomal recessive hypophosphatemic rickets, X-linked hypophosphatemic rickets, X-linked hypophosphatemia (XLH), etc.). The subpopulation is identified, for example, based on an elevated PPi concentration in such subjects. Causes of elevated PPi concentration include, for example, defects in signaling molecules, or mutations in genes that encode such signaling molecules, which regulate the production, degradation, or other ways influencing the stability of PPi. For example, the defects or mutations to signaling molecules may result in overexpression of PPi or decreased degradation or hydrolysis of PPi. HPP patients have defective or missing tissue nonspecific alkaline phosphatase, which can hydrolyze PPi. Thus, similar to HPP, in other diseases due to defects in alkaline phosphatases, PPi concentration may be elevated. The defects in signaling molecules also include defects in the co-factors or other molecules facilitating the function of the signaling molecules. For example, in CPPD, a deficiency of Mg, which acts as a cofactor for various phosphatases, leads to elevated levels of PPi.

Described herein are methods for identifying a subpopulation of subjects who either exhibit a muscle weakness disease-related symptom, or who are at risk for developing such muscle weakness disease-related symptoms. The identified population can include subjects who have previously been identified as having such muscle weakness disease or who are asymptomatic without a previous diagnosis. The muscle weakness diseases in the present disclosure include, for example, HPP or HPP-related diseases, CPPD or CPPD-related diseases, familial hypophosphatemia (such as autosomal dominant hypophosphatemic rickets (ADHR), autosomal recessive hypophosphatemic rickets, X-linked hypophosphatemic rickets, X-linked hypophosphatemia (XLH), etc.), or any other muscle weakness diseases with elevated PPi. Methods for identifying the subject subpopulation include, for example, the detection of elevated inorganic pyrophosphate (PPi) concentration in such subject.

Targeted Muscle Weakness Diseases

Provided herein are methods for treating or ameliorating at least one symptom of a subject having or being prone to a muscle weakness disease. The muscle weakness disease, myopathy, or myasthenia, described herein may include any disease or disorder which causes, is due to, or is related to at least one symptom of muscle weakness. The term "muscle weakness, "myopathy," "myasthenia" or other similar expressions in this disclosure refers to a condition related to impaired status of muscle function, such as a lack or defect of muscle strength, compared to other subjects having not such condition or to the same subject at the time point prior to having such condition. Muscle weakness can be divided into conditions that have either true or perceived muscle weakness. True muscle weakness may include a condition where the force exerted by the muscles is less than would be expected. For example, true muscle weakness includes a variety of skeletal muscle diseases, including muscular dystrophy and inflammatory myopathy. Exemplary disease or disorder includes neuromuscular junction disorders, such as

myasthenia gravis. Muscle weakness can also be caused by low levels of potassium and other electrolytes within muscle cells, where the force exerted by the muscles is less than would be expected. Perceived muscle weakness (or non-neuromuscular weakness) describes a condition where a subject feels more effort than normal (i.e., compared to other subjects having not such condition or to the same subject at the time point prior to having such condition) is required to exert a certain amount of force but actual muscle strength is normal, for example chronic fatigue syndrome.

In some conditions, such as myasthenia gravis, muscle strength is normal when resting, but true weakness occurs after the muscle has been subjected to exercise. This is also true for some cases of chronic fatigue syndrome, where objective post-exertion muscle weakness with delayed recovery time has been measured and is a feature of some of the published definitions. These diseases or disorders are also included in the "muscle weakness disease" of this disclosure.

Muscle weakness can also be classified as either "proximal" or "distal" based on the location of the muscles that it affects. Proximal muscle weakness affects muscles closest to the body's midline, while distal muscle weakness affects muscles further out on the limbs. Proximal muscle weakness can be seen in Cushing's Syndrome and Hyperthyroidism.

Other categories of muscle weakness exist in practice. For example, neuromuscular fatigue can be classified as either "central" or "peripheral" depending on its cause. Central muscle fatigue manifests as an overall sense of energy deprivation, while peripheral muscle fatigue manifests as a local, muscle-specific inability to do work

The severity of muscle weakness can be classified into different "grades" based on the following exemplary criteria:

Grade 0: No contraction or muscle movement.

Grade 1: Trace of contraction, but no movement at the joint.

Grade 2: Movement at the joint with gravity eliminated.

Grade 3: Movement against gravity, but not against added resistance.

Grade 4: Movement against external resistance with less strength than usual.

Grade 5: Normal strength.

Hypophosphatasia (HPP) and muscle weakness

Hypophosphatasia (HPP) is the rare inherited metabolic disorder resulting from loss-of-function mutation(s) in the tissue-nonspecific alkaline phosphatase (TNSALP) gene. The biochemical hallmark is subnormal ALP activity in serum (hypophosphatasemia), which leads to elevated blood and/or urine levels of three phosphocompound substrates: inorganic pyrophosphate (PPi), phosphoethanolamine (PEA) and pyridoxal 5'-phosphate (PLP). TNSALP deficiency can cause a spectrum of sequelae including premature loss of primary teeth, rickets, poor growth, muscle weakness, compromised physical function, and pain. Muscle weakness, or myopathy, has been found in association with HPP decades ago. For example, Seshia et al. (1990) reported that three children with HPP also had muscle pains, stiffness, and symptoms of proximal lower limb muscle weakness that occurred early in the disorder (remaining presenting in two of them). Interestingly, Seshia et al. (1990) found that those symptoms "could not be explained by skeletal impairment," but rather "resembled those in osteomalacia myopathy."

Other signs and symptoms with HPP may include: long-term pain in the muscles or joints, arthritis (in adults and children), pseudogout caused by deposits of calcium in the joints, inability to walk without an assistive device such as crutches, a walker, or a wheelchair, etc. Recently, asfotase alfa, a recombinant bone-targeted human TNASLP (i.e., sALP-Fc-D_{10}), has been reported to decrease the elevated inorganic pyrophosphate (PPi) concentration and improved skeletal mineralization, growth, and physical function of HPP patients. Children of 5-12-year old with HPP being treated with asfotase alfa for more than three years showed improvements in muscle strength, measured by Hand Held Dynamometry (HHD) and individual subtests of the Bruininks-Oseretsky Test of Motor Proficiency, 2nd Edition (BOT-2) including Strength and Running Speed/Agility scaled scores. As a result, they had significant gains in physical function of their muscles which impact ability to perform activities of daily living.

Asfotase alfa can be administered to treat, e.g., perinatal HPP, infantile HPP, childhood HPP, and odonto-HPP. For example, patients having childhood HPP (e.g., children of about 5 to about 12 years of age having HPP) or infantile HPP (e.g., infants of about 3 years of age or less than 3 years of age) can be treated with a sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) for a period of at least one year (e.g., at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or more than ten years (e.g., the lifetime of the patient)).

Since the asfotase alfa treatment dramatically improves bone mineralization in patients, it was uncertain whether its effects on patients' muscle strength was merely a result of the restored bone formation and the following restoration of skeletal muscle attachment/growth or a therapeutic effect directly to patients' muscles. As described herein, we have discovered that asfotase alfa has a therapeutic effect on the muscles of a patient (e.g., a patient having a muscle weakness disease, such as muscle weakness in a patient having HPP).

Calcium pyrophosphate deposition disease (CPPD, or CPDD) and muscle weakness

Calcium pyrophosphate deposition disease (CPPD, or CPDD), or calcium pyrophosphate dihydrate crystal deposition disease, is a metabolic arthropathy caused by the deposition of calcium pyrophosphate dihydrate crystals in and around joints, especially in articular cartilage and fibrocartilage. Although CPPD is often asymptomatic, with only radiographic changes seen (i.e., chondrocalcinosis), various clinical manifestations may occur, including acute (pseudogout) and chronic arthritis. The crystal deposits provoke inflammation in the joint, which can cause the joint cartilage to break down. The disease may take a few different arthritis-related forms: osteoarthritis, a chronic rheumatoid arthritis (RA)-like inflammatory arthritis, or an acutely painful inflammatory condition called pseudogout. The name pseudogout comes from the fact that it resembles another acutely painful condition called gout. The main difference is the type of crystals involved in the inflammation and damage. Almost any joint may be involved by CPPD, although the knees, wrists, and hips are most often affected. This condition is the most common cause of secondary metabolic osteoarthritis. Patients with CPPD can experience significant morbidity due to the pain of an acute attack of pseudogout or to symptoms of chronic

arthropathy. Treatment of symptomatic CPPD is important to prevent further end-organ damage, but it cannot reverse the joint disease.

The exact mechanism for the development of CPPD remains unclear. From aging, genetic factors, or both, patients have increased adenosine triphosphate breakdown resulting in increased inorganic pyrophosphate concentration in the joints. Changes in the cartilage matrix may play an important role in promoting calcium pyrophosphate dihydrate crystal deposition. Over activity of enzymes that break down triphosphates, such as nucleoside triphosphate pyrophosphohydrolase, has been observed in the cartilage of patients with CPPD. Therefore, inorganic pyrophosphate can bind calcium, leading to deposition in the cartilage and synovium. (see Beutler et al., 1993 *Arthritis Rheum.* 36(5):704-715). Hyaline cartilage is affected most commonly, but fibrocartilage, such as the meniscal cartilage of the knee, can also be involved. (Pritzker et al., 1988 *J Rheumatol.* 15(5):828-835).

Other diseases and muscle weakness

Similarly to HPP and CPPD (or CPDD), other diseases or disorders may include at least one symptom of muscle weakness. Among them, some types of muscle weakness diseases have characteristic elevated inorganic pyrophosphate (PPi) concentration. These muscle weakness diseases with elevated PPi concentration are also targets for treatment with asfotase alfa in the instant disclosure.

For example, familial hypophosphatemia (such as autosomal dominant hypophosphatemic rickets (ADHR), autosomal recessive hypophosphatemic rickets, X-linked hypophosphatemic rickets, X-linked hypophosphatemia (XLH), etc.) typically has a muscle weakness phenotype. Hypophosphatemia, or hypophosphatemic rickets, is a form of rickets that is characterized by low serum phosphate levels and resistance to treatment with ultraviolet radiation or vitamin D ingestion. X-linked hypophosphatemia (XLH) is a dominant disorder and accounts for more than 80% of all familial hypophosphatemia. XLH is considered to be a systemic disorder, from mutation of the phosphate-regulating gene homologous to endopeptidases on the X chromosome (PHEX). XLH patients demonstrate a normal or low serum concentration of 1,25-dihydroxyvitamin D3, suggestive of inadequate formation of this vitamin D metabolite. The remaining 20% of familial hypophosphatemia patients have autosomal dominant hypophosphatemic rickets from gain-of-function autosomal recessive hypophosphatemic rickets and hereditary hypophosphatemic rickets with hypercalciuria.

Methods of Treatment

Provided herein are methods for treating or ameliorating at least one symptom of a subject, child, adolescent, or adult, who has or is prone to a muscle weakness disease. Such treatment may include administering an alkaline phosphatase, or a polypeptide having alkaline phosphatase activity, to decrease the elevated PPi concentration in such subject. For example, a soluble alkaline phosphatase (sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) may be administered across a range of ages for children, adolescent, or adult subjects.

Subjects can be diagnosed with a muscle weakness disease (such as HPP, CPPD, familial hypophosphatemia described herein, etc.) prior to administration of an alkaline phosphatase, or a

polypeptide having alkaline phosphatase activity, (e.g., a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa). Additionally, a subject having or being prone to a muscle weakness disease can be a naïve subject that has not have previously received treatment with a sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa).

The method involves administering an alkaline phosphatase, or a polypeptide having alkaline phosphatase activity, (e.g., a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) to a subject having or being prone to a muscle weakness disease in a single or multiple dosages over a period of time. In particular, a sALP, such as asfotase alfa, can be administered to a subject previously determined to have elevated inorganic pyrophosphate (PPi) concentration or have at least one predetermined biomarker/score for muscle weakness, such as an average BOT-2 strength score of less than 10, an average BOT-2 running speed and agility score of less than 5, an average CHAQ index score greater than about 0.8, and/or an average PODCI score of less than about 40, an average 6MWT of less than about 80% of the predicted 6MWT value, a Muscle Strength Grade of less than 5, and/or an average HHD value (e.g., an average HHD muscle or grip strength value) of, e.g., less than about 80% of the predicted HHD value. For example, a sALP can be administered to a subject previously determined to have a concentration of PPi in a sample (e.g., a plasma sample) of greater than about 5.71 μM for an infant or child (e.g., a subject less than about 12 years of age); greater than about 4.78 μM for an adolescent (e.g., a subject of about 13 to about 18 years of age); or greater than about 5.82 μM for an adult (e.g., a subject of greater than about 18 years of age). In other embodiments, the muscle weakness disease described herein is caused by an elevated concentration of at least one alkaline phosphatase substrate (e.g., PPi, PLP, PEA, etc.). Alternatively, an alkaline phosphatase, or a polypeptide having alkaline phosphatase activity, (e.g. a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be administered to a subject having or being prone to a muscle weakness disease prior to determination of such scores (e.g., the BOT-2 strength score, BOT-2 running speed and agility score, the CHAQ index score, the BSID-III scaled score, the PDMS-2 standard score, a Muscle Strength score, a 6MWT value, and/or a HHD value) to allow for, e.g., an increase in activities of ADL, a decrease in pain, and/or improved motor development.

Additionally, each of the described scores (e.g., the BOT-2 strength score, BOT-2 running speed and agility score, the CHAQ index score, the BSID-III scaled score, the PDMS-2 standard score, 6MWT, the 12- POMA-G, a modified performance-oriented mobility assessment (mPOMA-G, such as the one illustrated in Phillips et al. 2015 Bone Abstracts 4:P136), or the HHD value) of a subject having or being prone to a muscle weakness disease described herein can be used singly or in any combination to assess treatment efficacy using a sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), in which improvements relative to a certain test score demonstrate that the sALP is effective for treating such muscle weakness disease.

For example, when administration of an alkaline phosphatase or a polypeptide having alkaline phosphatase activity (e.g., a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) to a subject having or being prone to a muscle weakness disease results in an average increase in the BOT-2 strength score to about 10 or greater than about 10, in which the subject previously had an average BOT-2 strength score of less than about 10, then the alkaline phosphatase or a polypeptide having alkaline phosphatase activity treatment is effective at treating, e.g., physical impairments associated with a muscle weakness disease. Alternatively, when administration of a sALP does not result in an average increase in the BOT-2 strength score to about 10 or greater than about 10, the dosage and/or frequency of alkaline phosphatase or a polypeptide having alkaline phosphatase activity administration can be changed in order to determine the effective amount of the alkaline phosphatase or a polypeptide having alkaline phosphatase activity for the subject. For instance, the dosage of the sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be increased from, e.g., about 3 mg/kg/week to about 6 mg/kg/week or about 6 mg/kg/week to about 9 mg/kg/wk.

Additionally, when administration of an alkaline phosphatase or a polypeptide having alkaline phosphatase activity (e.g., a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) to a subject having or being prone to a muscle weakness disease results in an improvement in the Muscle Strength Grade categorization of the subject of one or more (e.g., an improvement to a Muscle Strength Grade of 1, 2, 3, 4, or 5 from a prior, lower Muscle Strength Grade), in which the subject previously had an average Muscle Strength Grade of less than about 5, then the alkaline phosphatase or a polypeptide having alkaline phosphatase activity treatment is effective at treating, e.g., physical impairments associated with a muscle weakness disease. Alternatively, when administration of a sALP does not result in an improvement in the Muscle Strength Grade categorization of the subject of one or more from a prior, lower Muscle Strength Grade, the dosage and/or frequency of alkaline phosphatase or a polypeptide having alkaline phosphatase activity administration can be changed (e.g., increased) in order to determine the effective amount of the alkaline phosphatase or a polypeptide having alkaline phosphatase activity for the subject. For instance, the dosage of the sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be increased from, e.g., about 3 mg/kg/week to about 6 mg/kg/week or about 6 mg/kg/week to about 9 mg/kg/wk.

Biomarkers/Endpoints for Diagnosis and/or Treatment of Muscle Weakness Diseases

In preferred embodiments, a muscle weakness disease (such as HPP including, e.g., perinatal HPP, infantile HPP, childhood HPP, and odontohypophosphatasia, CPPD, and familial hypophosphatemia as described herein) is treated with an alkaline phosphatase, or a polypeptide having alkaline phosphatase activity, (e.g. a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g.,

asfotase alfa). The methods described herein are also useful for diagnosing a subject having or being prone to a muscle weakness disease, identifying a subject as a member in a specific subpopulation of subjects having or being prone to a muscle weakness disease, or testing the efficacy of treatment of a muscle weakness disease. For example, a subject may be diagnosed as having or being prone to a muscle weakness disease if such subject shows certain characteristic biomarkers. A subject may be treated with an alkaline phosphatase, or a polypeptide having alkaline phosphatase activity, (e.g. a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), while the treatment efficacy or effects may be analyzed using certain characteristic biomarkers or endpoints. Such biomarkers may include, e.g., the elevated inorganic pyrophosphate (PPi) concentration and/or the decreased alkaline phosphatase (ALP) in the serum, the bone or muscle tissues, or the urine of the subject. Exemplary endpoints useful in the methods described herein for muscle weakness treatment may include: (1) the Bruininks-Oseretsky Test of Motor Proficiency 2nd Edition (BOT-2), (2) the Childhood Health Assessment Questionnaire (CHAQ), (3) the Pediatric Outcomes Data Collection Instrument (PODCI), (4) Bayley Scales of Infant and Toddler Development, 3rd Edition (BSID-III), (5) the Peabody Developmental Motor Scales, 2nd Edition (PDMS-2), (6) the Six Minute Walk Test (6MWT), (7) the Muscle Strength Grade, and (8) Handheld Dynamometry (HHD), which are described in further detail below.

Plasma Inorganic Pyrophosphate (PPi) and Alkaline Phosphatase (ALP) Concentrations

Subjects having or being prone to a muscle weakness disease can be identified for treatment with an alkaline phosphatase or a polypeptide having alkaline phosphatase activity, (e.g. a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) by determining the inorganic pyrophosphate (PPi) and/or alkaline phosphatase (ALP) concentrations in a sample, such as a plasma or urine sample, from the patient. Any method known to those of skill in the art can be used to quantify the PPi and/or ALP concentrations in a plasma sample or alternatively in a urine sample, as described in detail in Whyte et al., 1995 (J. Clin. Invest. 95(4): 1440–1445), hereby incorporated by reference in its entirety. Methods to quantify PPi concentrations in a plasma or urine sample are also described in Cheung et al., 1977 (Anal. Biochem. 83: 61-63), Cook et al., 1978 (Anal. Biochem. 91: 557-565), and Johnson et al, 1968 (Anal. Biochem. 26: 137-145), which are each hereby incorporated by reference in their entirety.

In particular, an alkaline phosphatase or a polypeptide having alkaline phosphatase activity (e.g. a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be administered to a subject (e.g., a human) having or being prone to a muscle weakness disease previously determined to have a plasma PPi concentration of up to about 6 μM (e.g., about 4.5 μM, about 5 μM, or about 5.5 μM or a plasma PPi concentration within the range of about 4.5 μM to about 6 μM). For example, the alkaline phosphatase or the polypeptide having alkaline phosphatase activity is administered to, e.g., an infant or child (e.g., a subject less than about 12 years of age) having a plasma

PPi concentration of about 5.71 μM or greater; an adolescent (e.g., a subject of about 13 to about 18 years of age) having a plasma PPi concentration of about 4.78 μM or greater; or an adult (e.g., a subject of greater than about 18 years of age) having a plasma PPi concentration of about 5.82 μM or greater. Additionally, an alkaline phosphatase or a polypeptide having alkaline phosphatase activity can be administered to a subject (e.g., a human) having or being prone to a muscle weakness disease previously determined to have a plasma ALP concentration of, e.g., about 90 U/L or less for a subject of 0 to 14 days of age; about 134 U/L or less for a subject of 15 days of age to less than 1 year of age; about 156 U/L or less for a subject of about 1 year of age to less than 10 years of age; about 141 U/L or less for a subject of about 10 years of age to less than about 13 years of age; about 62 U/L or less for a female subject of about 13 years of age to less than about 15 years of age; about 127 U/L or less for a male subject of about 13 years of age to less than about 15 years of age; about 54 U/L or less for a female subject of about 15 years of age to less than about 17 years of age; about 89 U/L or less for a male subject of about 15 years of age to less than about 17 years of age; about 48 U/L or less for a female subject of about 17 years of age or older; or about 59 U/L or less for a male subject of about 17 years of age or older.

The plasma PPi concentration and/or plasma ALP concentration of a subject (e.g., a human) having or being prone to a muscle weakness disease can be compared to the plasma PPi concentration and/or plasma ALP of a normal subject to determine a treatment effect in the subject administered an alkaline phosphatase or a polypeptide having alkaline phosphatase activity (e.g. a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa). In particular, the alkaline phosphatase or the polypeptide having alkaline phosphatase activity can be administered for a treatment period of least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient). Alternatively, the methods can include determining the plasma PPi concentration and/or plasma ALP concentration prior to administering the alkaline phosphatase or the polypeptide having alkaline phosphatase activity to assess an effect in the subject of treatment with the alkaline phosphatase or the polypeptide having alkaline phosphatase activity.

The methods result in a decrease in PPi and/or an increase in ALP concentration in a sample (e.g., a plasma sample) from a subject (e.g., a human) having or being prone to a muscle weakness disease. For example, treatment with an alkaline phosphatase or a polypeptide having alkaline phosphatase activity (e.g. a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) results in a decrease in PPi concentration in a sample (e.g., a plasma sample) from the patient of about 1μM, about 1.5 μM, about 2 μM, about 2.5 μM, or about 3 μM or 25% or greater (e.g., 30%, 35%, 40%, 45%, 50%, 55%, 60%, or more than 60%). Thus, the subject exhibits a plasma PPi concentration of, e.g., about 2 μM to about 5 μM, about 3 μM to about 5 μM, about 2 μM to about 4 μM, or about 2 μM to about 3 μM after administration of the alkaline phosphatase or the polypeptide having alkaline phosphatase activity.

Likewise, treatment with alkaline phosphatase or a polypeptide having alkaline phosphatase activity results in an increase in ALP concentration in a sample (e.g., a plasma sample) from a subject (e.g., a human) having or being prone to a muscle weakness disease of 30%, 35%, 40%, 45%, 50%, 55%, 60%, or more than 60%, relative to the subject prior to administration of the alkaline phosphatase or a polypeptide having alkaline phosphatase activity. For example, administration of the alkaline phosphatase or the polypeptide having alkaline phosphatase activity increases the ALP concentration in a sample (e.g., a plasma sample) from the subject to, e.g, about 273 U/L or greater for a subject of 0 to 14 days of age; about 518 U/L or greater for a subject of 15 days of age to less than 1 year of age; about 369 U/L or greater for a of about 1 year of age to less than 10 years of age; about 460 U/L or greater for a subject of about 10 years of age to less than about 13 years of age; about 280 U/L or greater for a female subject of about 13 years of age to less than about 15 years of age; about 517 U/L or greater for a male subject of about 13 years of age to less than about 15 years of age; about 128 U/L or greater for a female subject of about 15 years of age to less than about 17 years of age; about 365 U/L or greater for a male subject of about 15 years of age to less than about 17 years of age; about 95 U/L or greater for a female subject of about 17 years of age or older; or about 164 U/L or greater for a male subject of about 17 years of age or older.

The decrease in the plasma PPi and/or increase in the ALP concentrations of the subject (e.g., a human) having or being prone to a muscle weakness disease can be sustained throughout administration of the alkaline phosphatase or the polypeptide having alkaline phosphatase activity (e.g. a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa). For instance, the plasma PPi concentration decreases by about 25% and remains at ± 10% of the decreased plasma PPi concentration during treatment with the sALP and/or the plasma ALP concentration increases by about 50% and remains at ± 10% of the increased plasma ALP concentration during treatment with the alkaline phosphatase or the polypeptide having alkaline phosphatase activity.

Alternatively, when administration of an alkaline phosphatase or a polypeptide having alkaline phosphatase activity (e.g. a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) does not result in an average decrease in PPi concentrations in a plasma sample from the subject (e.g., a human) having or being prone to a muscle weakness disease by about 25% or greater, the dosage and/or frequency of sALP administration can be changed in order to determine the effective amount of the sALP for the subject. Likewise, when administration of an alkaline phosphatase or a polypeptide having alkaline phosphatase activity does not result in an average increase in ALP concentrations in a plasma sample from the subject by about 50% or greater, the dosage and/or frequency of alkaline phosphatase or a polypeptide having alkaline phosphatase activity administration can be changed in order to determine the effective amount of the an alkaline phosphatase or a polypeptide having alkaline phosphatase activity for the subject. For instance, the dosage of the an alkaline phosphatase or a polypeptide having alkaline phosphatase activity can be increased from, e.g., about 2.1 mg/kg/week or about 3.5 mg/kg/week to about 6 mg/kg/week or about 9 mg/kg/week.

Bruininks-Oseretsky Test of Motor Proficiency 2nd Edition (BOT-2)

An exemplary Bruininks-Oseretsky Test of Motor Proficiency 2nd Edition (BOT-2) is described in Bruininks, R. H. (2005). *Bruininks-Oseretsky Test of Motor Proficiency, (BOT-2)*, Minneapolis, MN: Pearson Assessment, hereby incorporated by reference in its entirety. In particular, the BOT-2 can be used to evaluate physical impairments and mobility restrictions in a subject having or being prone to a muscle weakness disease (e.g., HPP) to generate a BOT-2 score for the subject.

The BOT-2 includes a range of tests to evaluate physical impairments of a subject, which can be performed with, e.g., a kit including the tests. The BOT-2 provides composite BOT-2 scores in the following areas: strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and upper-limb coordination. For example, a subject having or being prone to a muscle weakness disease can perform sit-ups, v-ups, standing long jump, wall sit, and/or push-ups to determine the BOT-2 strength score.; a subject having or being prone to a muscle weakness disease can step over a balance beam and/or perform a shuttle run, two-legged side hop, and/or one-legged side hop to determine the BOT-2 running speed and agility score; a subject having or being prone to a muscle weakness disease can cut out a circle and/or connect dots to determine the BOT-2 fine motor precision score; a subject having or being prone to a muscle weakness disease can copy a star and/or copy a square to determine the BOT-2 fine motor integration score; a subject having or being prone to a muscle weakness disease can transfer pennies, sort cards, and/or string blocks to determine the manual dexterity score; a subject having or being prone to a muscle weakness disease can tap his or her foot and finger and/or perform jumping jacks to determine the BOT-2 bilateral coordination score; a subject having or being prone to a muscle weakness disease can walk forward on a line and/or stand on one leg on a balance beam to determine the BOT-2 balance score; and a subject having or being prone to a muscle weakness disease can throw a ball at a target and/or catch a tossed ball to determine the BOT-2 upper-limb coordination score.

A subject having or being prone to a muscle weakness disease (e.g., HPP) could perform tests in one or more of described areas (strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and upper-limb coordination) to generate a BOT-2 score indicative of physical impairments in the subject. Within each BOT-2 area (strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and upper-limb coordination), such subject could perform one or more tests to determine the BOT-2 score of the subject, e.g., the subject could perform one or more of sit-ups, v-ups, standing long jump, wall sit, and push-ups to determine the BOT-2 strength score. Thus, only one test (e.g., one test selected from the group of sit-ups, v-ups, standing long jump, wall sit, and push-ups) can be performed to determine the BOT-2 score (e.g., a BOT-2 strength score) of a subject having or being prone to a muscle weakness disease (e.g., HPP).

Each of the BOT-2 scores (strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and upper-limb coordination) of the subject having or being prone to a muscle weakness disease (e.g., HPP) can be compared to the BOT-2 score of a subject without the muscle weakness disease (e.g., HPP) to, e.g., determine the standard deviation of the BOT-2 score. Each of the BOT-2 scores (e.g., strength, running speed and agility, fine motor

precision, fine motor integration, manual dexterity, bilateral coordination, balance, and upper-limb coordination) of the subject having or being prone to a muscle weakness disease (e.g., HPP) can be compared to the BOT-2 score of other subjects having or being prone to the muscle weakness disease (e.g., HPP) to, e.g., determine the average BOT-2 score for the subject.

BOT-2 scores (e.g., strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and upper-limb coordination scores) range from about 0 to equal to or less than about 25, in which a score of about 10 to about 20 is considered representative of healthy subject (e.g., subject without the muscle weakness disease (e.g., HPP)). Subjects with an average BOT-2 score (e.g., strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and upper-limb coordination scores) of less than about 10 can be treated with an alkaline phosphatase, or a polypeptide having alkaline phosphatase activity, e.g., sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa.

For example, subjects having or being prone to a muscle weakness disease with a BOT-2 strength score of less than 10 (e.g., about 0, about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10) can be treated with a sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) for a period of time, up to the lifetime of the patient. Likewise, subjects having or being prone to a muscle weakness disease with a BOT-2 running speed and agility score of less than 10 (e.g., about 0, about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10) can then be treated with a sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) for a period of time, up to the lifetime of the subject.

The methods can result in an improvement in the BOT-2 score (e.g., strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and/or upper-limb coordination score) of a subject having or being prone to a muscle weakness disease (e.g., HPP). For example, treatment with an alkaline phosphatase, or a polypeptide having alkaline phosphatase activity, such as a sALP (e.g., TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), such as treatment with a sALP for a period of time, can result in an average increase in the BOT-2 strength score to about 10 to about 20 (e.g. about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, or about 20). Additionally, treatment with a sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can result in an average increase in the BOT-2 running speed and agility score to about 5 to about 20 (e.g. about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, or about 20).

The increase in the BOT-2 score (e.g., strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and/or upper-limb coordination

score) can be sustained throughout administration of the alkaline phosphatase, or the polypeptide having alkaline phosphatase activity, such as sALP (e.g., TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), e.g., for a period of time. Likewise, the decrease in physical impairments of muscles after administration of the alkaline phosphatase, or the polypeptide having alkaline phosphatase activity, can be sustained throughout administration of the alkaline phosphatase, or the polypeptide having alkaline phosphatase activity.

The BOT-2 scores (strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and upper-limb coordination scores) of a subject having or being prone to a muscle weakness disease (such as, HPP) can be used singly or in combination to other endpoints for assessing treatment efficacy using an alkaline phosphatase, or a polypeptide having alkaline phosphatase activity, such as sALP (e.g., TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), in which improvements relative to a certain test score demonstrate that the alkaline phosphatase, or the polypeptide having alkaline phosphatase activity, is effective for treating muscle impairments associated with the muscle weakness disease. For example, when administration of a sALP to a subject having or being prone to a muscle weakness disease results in an average increase in the BOT-2 running speed and agility score to about 5 or greater than about 5, in which the subject previously had an average BOT-2 running speed and agility score of less than about 5, then the sALP is considered to be effective at, e.g., treating physical impairments associated with a muscle weakness disease.

Additionally, within each BOT-2 area (strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and upper-limb coordination), a subject having or being prone to a muscle weakness disease (e.g., HPP, CPPD, familial hypophosphatemia described herein, etc.) could perform one or more tests to determine the BOT-2 score of the subject. For instance, a subject having or being prone to a muscle weakness disease could perform one or more of sit-ups, v-ups, standing long jump, wall sit, and push-ups to determine the BOT-2 strength score, to determine the BOT-2 strength score and assess the treatment efficacy of sALP administration. The subject having or being prone to a muscle weakness disease can perform one or more of balance beam, a shuttle run, two-legged side hop, and/or one-legged side hop to determine the BOT-2 running speed and agility score and assess the treatment efficacy of sALP administration. The subject having or being prone to a muscle weakness disease can cut out a circle and/or connect dots to determine the BOT-2 fine motor precision score and assess the treatment efficacy of sALP administration. The subject having or being prone to a muscle weakness disease can copy a star and/or copy a square to determine the BOT-2 fine motor integration score and assess the treatment efficacy of sALP administration. The subject having or being prone to a muscle weakness disease could perform one or more of transferring pennies, sorting cards, and stringing blocks to determine the BOT-2 manual dexterity score and assess the treatment efficacy of sALP administration. The subject having or being prone to a muscle weakness disease can tap his or her foot and finger and/or perform jumping jacks to determine the BOT-2 bilateral coordination score and assess the treatment efficacy of sALP

administration. The subject having or being prone to a muscle weakness disease can walk forward on a line and/or stand on one leg on a balance beam to determine the BOT-2 balance score and assess the treatment efficacy of sALP administration. The subject having or being prone to a muscle weakness disease can throw a ball at a target and/or catch a tossed ball to determine the BOT-2 upper-limb coordination score and assess the treatment efficacy of sALP administration.

Alternatively, when administration of an alkaline phosphatase, or a polypeptide having alkaline phosphatase activity, such as a sALP, does not result in an average increase in the BOT-2 running speed and agility score to greater than about 5, the dosage and/or frequency of administration can be changed in order to determine the effective amount of the alkaline phosphatase, or the polypeptide having alkaline phosphatase activity, for the subject having or being prone to the muscle weakness disease (e.g., HPP). For instance, the dosage of the sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be increased from, e.g., about 3 mg/kg/week to about 6 mg/kg/week or about 6 mg/kg/week to about 9 mg/kg/wk.

Childhood Health Assessment Questionnaire (CHAQ)

The Childhood Health Assessment Questionnaire (CHAQ) can be administered to evaluate the health status of children having a muscle weakness disease (e.g., HPP) to generate a CHAQ index score for the child, as is described in Bruce & Fries (*J. Rheumatol.* 30(1): 167-178, 2003) and Klepper (*Arthritis & Rheumatism*, 49: S5–S14, 2003), hereby incorporated by reference in their entirety. The CHAQ includes eight categories of questions for dressing/grooming, arising, eating, walking, hygiene, reach, grip, and activities, in which a parent or guardian records the amount of difficulty the child with the muscle weakness disease (e.g., HPP) has in performing the respective activities. The range of scores within each category is from 0 to 3, in which a score of 0 indicates without any difficulty; a score of 1 indicates with some difficulty; a score of 2 indicates with much difficulty; and a score of 3 indicates that the child is unable to perform the activity.

Children having or being prone to a muscle weakness disease with an average CHAQ index score (e.g., indicative of disability in activities of daily living (ADL) and/or pain) greater than about 0.8 (e.g., about 0.8, about 1, about 1.2, about 1.4, about 1.6, about 1.8, about 2.0, about 2.2, about 2.4, about 2.6, about 2.8, or about 3.0) can be treated by administering an alkaline phosphatase, or a polypeptide having alkaline phosphatase activity, such as sALP (e.g., TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa). For example, children with an average CHAQ index score of greater than about 0.8 can be treated by administering an alkaline phosphatase, or a polypeptide having alkaline phosphatase activity, such as sALP (e.g., TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) for a period of time, up to the lifetime of the patient. Furthermore, a child having or being prone to a muscle weakness disease disclosed herein could be asked one or more questions in one or more of the eight categories (dressing/grooming, arising, eating, walking, hygiene, reach, grip, and activities) to arrive at an average CHAQ index score, and if the average CHAQ index score is greater

than about 0.8, the child can be treated by administering an alkaline phosphatase, or a polypeptide having alkaline phosphatase activity, such as a sALP.

The CHAQ index score of a child having or being prone to a muscle weakness disease disclosed herein can be compared to the CHAQ index score of children without such muscle weakness disease to, e.g., determine the standard deviation of the CHAQ index score. Additionally, the CHAQ index score of a child having or being prone to a muscle weakness disease disclosed herein can be compared to the CHAQ index score of other children having or being prone to the muscle weakness disease disclosed herein to, e.g., determine the standard deviation of the CHAQ index score.

The methods can result in an improvement in the CHAQ index score (e.g., indicative of disability in ADL and/or pain) of the child having or being prone to a muscle weakness disease disclosed herein. For example, treatment with a sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), such as treatment with a sALP for a period of time, up to the lifetime of the child, can result in an average decrease in the CHAQ index score to about 0 to equal to or less than about 0.5 (e.g. about 0, about 0.1, about 0.2, about 0.4, or about 0.5) in children with HPP.

The decrease in the CHAQ index score of the child having or being prone to a muscle weakness disease (e.g., HPP) can be sustained throughout administration of the alkaline phosphatase, or the polypeptide having alkaline phosphatase activity, such as sALP (e.g., TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), e.g., for a period of time, up to the lifetime of the child. Likewise, the increase in ADL and/or decrease in pain of the child can be sustained throughout administration of the sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), for a period of time, up to the lifetime of the child.

The CHAQ index score of a child having or being prone to a muscle weakness disease (e.g., HPP) can be used to assess treatment efficacy using an alkaline phosphatase, or a polypeptide having alkaline phosphatase activity, such as sALP (e.g., TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), in which improvements relative to a certain test score demonstrate that the alkaline phosphatase, or the polypeptide having alkaline phosphatase activity, is effective for treating, e.g., disability in activities of daily living (ADL) and pain associated with the muscle weakness disease. In particular, a child having or being prone to a muscle weakness disease could be asked one or more questions in one or more of the eight categories (dressing/grooming, arising, eating, walking, hygiene, reach, grip, and activities) to arrive at an average CHAQ index score and to assess treatment efficacy of sALP administration. For example, when administration of a sALP to a child having or being prone to a muscle weakness disease results in an average decrease in the CHAQ index score to equal to or less than about 0.5, in which the child previously had an average CHAQ index score of greater than about 0.8, then the sALP is effective at treating, e.g., disability in activities of daily living (ADL) and pain associated with a muscle weakness disease. Alternatively, when administration of a sALP does not result in an average decrease in the CHAQ index score to equal to or less than about 0.5, the dosage and/or

frequency of sALP administration can be changed in order to determine the effective amount of the sALP for the child having or being prone to a muscle weakness disease. For instance, the dosage of the sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be increased from, e.g., about 3 mg/kg/week to about 6 mg/kg/week or about 6 mg/kg/week to about 9 mg/kg/wk.

Pediatric Outcomes Data Collection Instrument (PODCI)

Certain subjects having or being prone to a muscle weakness disease (e.g., HPP) can be identified for treatment with an alkaline phosphatase, or a polypeptide having alkaline phosphatase activity, such as sALP (e.g., TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) using the Pediatric Outcomes Data Collection Instrument (PODCI). The PODCI can be administered to evaluate the health status of children to generate a PODCI score for the patient, as is described in Plint et al. (*J. Pediatr. Orthop.* *23*(6): 788-790, 2003). The PODCI includes eight categories of questions that can be completed by a subject having or being prone to a muscle weakness disease (e.g., HPP) or by a parent/guardian of the subject. Categories that can be used to determine the PODCI of a subject having or being prone to a muscle weakness disease include the following: 1) the upper extremity and physical function scale to measure difficulty encountered in performing daily personal care and student activities; 2) the transfer and basic mobility scale to measure difficulty experienced in performing routine motion and motor activities in daily activities; 3) the sports/physical functioning scale to measure difficulty or limitations encountered in participating in more active activities or sports; 4) the pain/comfort scale to measure the level of pain experienced during the past week; 5) the treatment expectations scale to measure the long term expectations of treatment; 6) the happiness scale to measure overall satisfaction with personal looks and sense of similarity to friends and others of own age; 7) the satisfaction with symptoms scale to measure the patient's acceptance of current limitations should this be a life-long state; and 8) the global functioning scale, which is a general combined scale calculated from the first four scales listed above. In each of the categories, a standardized score is determined for the subject having or being prone to a muscle weakness disease and then converted to a 0 to 100 scale, in which 0 represents significant disability and 100 represents less disability.

Subjects having or being prone to a muscle weakness disease (e.g., HPP) with an average PODCI score (e.g., indicative of disability in ADL and/or pain) less than about 40 (e.g., about 5, about 10, about 15, about 20, about 25, about 30, about 35, or about 39) can be treated by administering an alkaline phosphatase, or a polypeptide having alkaline phosphatase activity, such as a sALP (e.g., TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa). For example, subjects with an average PODCI score of less than 40 can be treated by administering a sALP for a period of time, up to the lifetime of the patient. Furthermore, a subject having or being prone to a muscle weakness disease could be asked one or more questions in one or more of the eight scales described above (e.g., transfer and basic mobility, sports/physical functioning, and the pain/comfort scale) to arrive at an average PODCI

score, and if the average PODCI score is greater than less than 40, the patient can be treated by administering a sALP.

The methods described herein can result in an increase in the PODCI score (e.g., indicative of disability in ADL and/or pain) of the subject having or being prone to a muscle weakness disease. For example, treatment with an alkaline phosphatase, or a polypeptide having alkaline phosphatase activity, such as a sALP (e.g., TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), such as treatment with a sALP for a period of time, up to the lifetime of the subject, can result in an average increase in the PODCI score to about 40 to about 50 (e.g. about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, or about 50).

The increase in the PODCI score can be sustained throughout administration of the alkaline phosphatase, or the polypeptide having alkaline phosphatase activity, such as the sALP (e.g., TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), e.g., for a period of time, up to the lifetime of the subject having or being prone to a muscle weakness disease. Likewise, the increase in ADL and/or decrease in pain can be sustained throughout administration of the sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), for a period of time, up to the lifetime of the subject.

The PODCI score of a subject having or being prone to a muscle weakness disease (e.g., HPP) can be used to assess treatment efficacy using an alkaline phosphatase, or a polypeptide having alkaline phosphatase activity, such as a sALP (e.g., TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), in which improvements relative to a certain test score demonstrate that the alkaline phosphatase, or the polypeptide having alkaline phosphatase activity, is effective for treating, e.g., disability in activities of daily living (ADL) and pain associated with the muscle weakness disease. In particular, a subject having or being prone to a muscle weakness disease could be asked one or more questions in one or more of the eight scales (the upper extremity and physical function scale, the transfer and basic mobility scale, the sports/physical functioning scale, the pain/comfort scale, the treatment expectations scale, the happiness scale, the satisfaction with symptoms scale, and the global functioning scale) to arrive at an average PODCI score and to assess treatment efficacy of sALP administration.

For example, when administration of a sALP to a subject having or being prone to a muscle weakness disease results in an average increase in the PODCI score to about 40 or greater than about 40, in which the subject previously had an average PODCI score of less than about 40, then the sALP is effective at treating, e.g., disability in activities of daily living (ADL) and pain associated with a muscle weakness disease. Alternatively, when administration of a sALP does not result in an average increase in the PODCI score to about 40 or greater than about 40, the dosage and frequency of sALP administration can be changed in order to determine the effective amount of the sALP for the subject having or being prone to a muscle weakness disease. For instance, the dosage of the sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95%

sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be increased from, e.g., about 3 mg/kg/week to about 6 mg/kg/week or about 6 mg/kg/week to about 9 mg/kg/wk.

Bayley Scales of Infant and Toddler Development, 3rd Edition (BSID-III)

Another endpoint, the Bayley Scales of Infant and Toddler Development, 3rd Edition (BSID-III) can be administered to evaluate the health status of a subject having or being prone to a muscle weakness disease (e.g., HPP) from birth to generate a BSID-III score for the subject, as is described in Bayley. (2006). *Bayley scales of infant and toddler development: administration manual*. San Antonio, TX: Harcourt Assessment. The BSID-III includes a series of developmental play tasks that can be administered to the subject to determine the raw BSID-III score. For example, categories for determining the BSID-III score of a subject having or being prone to a muscle weakness disease (e.g., infants of about three years of age or less having HPP) can include prehension, perceptual-motor integration, motor planning and speed, visual tracking, reaching, object grasping, object manipulation, functional hand skills, responses to tactile information, movement of the limbs and torso, static positioning, dynamic movement, balance, and motor planning. The BSID-III measurements are then converted to scaled BSID-III scores, which can be used to determine the subject's performance compared to healthy, age-adjusted subjects. The BSID-III scaled score of a subject having or being prone to a muscle weakness disease (e.g., a patient with HPP) can range from 0 to 14, in which scores of about 7 to about 13 are considered the normal range of healthy subjects.

A subject having or being prone to a muscle weakness disease could perform tests in one or more of described categories (prehension, perceptual-motor integration, motor planning and speed, visual tracking, reaching, object grasping, object manipulation, functional hand skills, responses to tactile information, movement of the limbs and torso, static positioning, dynamic movement, balance, and motor planning) as an infant (e.g., at about 3 years of age or less than 3 years of age) to generate a BSID-III score indicative of delayed motor development. Subjects having or being prone to a muscle weakness disease with an average BSID-III score in one or more of the described categories (prehension, perceptual-motor integration, motor planning and speed, visual tracking, reaching, object grasping, object manipulation, functional hand skills, responses to tactile information, movement of the limbs and torso, static positioning, dynamic movement, balance, and motor planning) less than about 2 as an infant can be treated by administering a sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa). In particular, subjects having or being prone to a muscle weakness disease with an average BSID-III score of less than about 2 as an infant can be treated by administering a sALP for a period of time, up to the lifetime of the subject.

The methods can result in an improvement in the average BSID-III score (e.g., indicative of delayed motor development) of the subject having or being prone to a muscle weakness disease. For example, treatment with a sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), such as treatment with a sALP for a period of time, up to the lifetime of the subject, can

result in an average increase in the BSID-III score to greater than about 5 (e.g., about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, or about 13).

The increase in the BSID-III score can be sustained throughout administration of the alkaline phosphatase, or the polypeptide having alkaline phosphatase activity, such as a sALP (e.g., TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), for a period of time, up to the lifetime of the subject having or being prone to a muscle weakness disease. Likewise, the increase in motor development can be sustained throughout administration of the alkaline phosphatase, or the polypeptide having alkaline phosphatase activity, such as a sALP (e.g., TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), e.g., for a period of time, up to the lifetime of the subject.

The BSID-III score of a subject having or being prone to a muscle weakness disease (e.g., HPP) can be used to assess treatment efficacy using an alkaline phosphatase, or a polypeptide having alkaline phosphatase activity, such as a sALP (e.g., TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), in which improvements relative to a certain test score demonstrate that the alkaline phosphatase, or the polypeptide having alkaline phosphatase activity, is effective for treating, e.g., delayed motor development associated with the muscle weakness disease. In particular, a subject having or being prone to a muscle weakness disease could perform tests in one or more of described categories (prehension, perceptual-motor integration, motor planning and speed, visual tracking, reaching, object grasping, object manipulation, functional hand skills, responses to tactile information, movement of the limbs and torso, static positioning, dynamic movement, balance, and motor planning) as an infant (e.g., at about three years of age or less having HPP) to arrive at an average BSID-III score and to assess treatment efficacy of sALP administration.

For example, when administration of a sALP to a child having or being prone to a muscle weakness disease results in an average increase in the BSID-III scaled score to greater than about 5, in which the child previously had an average BSID-III scaled score of less than about 2 as an infant (e.g., at about 3 years of age or less than 3 years of age), then the sALP is effective at treating, e.g., delayed motor development associated with HPP. Alternatively, when administration of a sALP does not result in an average increase in the BSID-III scaled score to greater than about 5, the dosage and/or frequency of sALP administration can be changed in order to determine the effective amount of the sALP for the child having or being prone to a muscle weakness disease. For instance, the dosage of the sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be increased from, e.g., about 3 mg/kg/week to about 6 mg/kg/week or about 6 mg/kg/week to about 9 mg/kg/wk.

Peabody Developmental Motor Scales, 2nd Edition (PDMS-2)

Another endpoints, the Peabody Developmental Motor Scales, 2nd Edition (PDMS-2), can be administered to evaluate the health status of a subject having or being prone to a muscle weakness disease (e.g., HPP) from birth to generate a PDMS-2 score for the subject, as is described in van

Hartingsveldt et al. (*Occup. Ther. Int.* 12(1): 1-13, 2005). The PDMS-2 includes six categories of subtests to measure motor skills of the subject, such as a patient having HPP.

In particular, PDMS-2 measurements can be determined from the following subtests: 1) the locomotor subtest to measure a subject's ability to move from one place to another (measurements include crawling, walking, running, hopping, and jumping forward); 2) the reflexes subtest to measure a subject's ability to automatically react to environmental events; 3) the stationary subtest to measure a subject's ability to sustain body control within the center of gravity and retain equilibrium; 4) the object manipulation subtest to measure a subject's ability to manipulate an object, such as catching, throwing, and kicking a ball; 5) the grasping subtest to measure a subject's ability to use his or her hands, such as the ability to hold an object with one hand and actions involving the controlled use of the fingers of both hands; and 6) the visual-motor integration subtest to measure a subject's ability to use his or her visual perceptual skills to perform complex eye-hand coordination tasks, such as reaching and grasping for an object, building with blocks, and copying designs. The PDMS-2 measurement can be determined for one or more of these categories for a subject having or being prone to a muscle weakness disease (e.g., HPP) and then converted into a PDMS-2 score, such as the PDMS-2 locomotor standard score ranging from 0 to 13, in which the range of healthy subjects (e.g., subjects without the muscle weakness disease) is from about 7 to about 13.

Subjects having or being prone to a muscle weakness disease with an average PDMS-score (e.g., indicative of delayed motor development) can be treated by administering a sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa).

The methods described herein can result in an improvement in the PDMS-2 score (e.g., indicative of delayed motor development) of the subject having or being prone to a muscle weakness disease. For example, treatment with an alkaline phosphatase, or a polypeptide having alkaline phosphatase activity, such as a sALP (e.g., TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), can result in an average increase in the PDMS-2 score to about 7 to about 13 (e.g., about 7, about 8, about 9, about 10, about 11, about 12, or about 13).

The increase in the PDMS-2 score can be sustained throughout administration of the alkaline phosphatase, or the polypeptide having alkaline phosphatase activity, such as sALP (e.g., TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), for an elongated time, e.g., for a period of time, up to the lifetime of the subject having or being prone to a muscle weakness disease. Likewise, the increase in motor development can be sustained throughout administration of the sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) for a period of time, up to the lifetime of the subject having or being prone to a muscle weakness disease.

The PDMS-2 score of a subject having or being prone to a muscle weakness disease (e.g., HPP) can be used to assess treatment efficacy using an alkaline phosphatase, or a polypeptide having alkaline phosphatase activity, such as a sALP (e.g., TNALP, for example the sALP polypeptide of SEQ ID NO: 1

or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), in which improvements relative to a certain test score demonstrate that the alkaline phosphatase, or the polypeptide having alkaline phosphatase activity, is effective for treating, e.g., delayed motor development associated with the muscle weakness disease. For example, a child having or being prone to a muscle weakness disease could perform tests in one or more of described categories (locomotor, reflexes, stationary, object manipulation, grasping, and visual-motor) at about 5 years of age or less than 5 years of age to arrive at an average PDMS-2 score and to assess treatment efficacy of sALP administration.

For example, when administration of a sALP to a child having or being prone to a muscle weakness disease results in an average increase in the PDMS-2 standard score to about 7, in which the child previously had an average PDMS-2 standard score of about 5, then the sALP is effective at treating, e.g., delayed motor development associated with HPP. Alternatively, when administration of a sALP does not result in an average increase in the PDMS-2 standard score to about 7, the dosage and/or frequency of sALP administration can be changed in order to determine the effective amount of the sALP for the child. For instance, the dosage of the sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be increased from, e.g., about 3 mg/kg/week to about 6 mg/kg/week or about 6 mg/kg/week to about 9 mg/kg/wk.

Six Minute Walk Test (6MWT)

A subject having a muscle weakness disease can be identified for treatment with an alkaline phosphatase or a polypeptide having alkaline phosphatase activity (e.g. a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) using the 6MWT. In particular, the 6MWT can be used to evaluate walking ability in an adult having a muscle weakness disease to generate a 6MWT value for the adult. The 6MWT can be performed indoors or outdoors using a flat, straight, enclosed corridor (e.g., of about 30 meters in length) with a hard surface. A stopwatch or other timer can be used to track the time and a mechanical counter or other device can be used to determine the distance (e.g., in meters) that the subject having a muscle weakness disease walks. For instance, the length of the corridor can be marked every three meters to determine the number of meters walked by the subject having a muscle weakness disease, with the turnaround point at 30 meters and the starting line also marked. The distance walked by the subject having a muscle weakness disease in six minutes can then be compared to the predicted number of meters walked, e.g., by a normal subject of about the same age, the same gender, and/or the same height, and expressed as a percentage value to generate the 6MWT value of the subject. The 6MWT value of the subject having a muscle weakness disease can be compared to the 6MWT value at baseline of the subject. Additionally, the 6MWT value of the subject having a muscle weakness disease can be compared to the 6MWT value of a normal subject.

Subjects having a muscle weakness disease with an average 6MWT of less than about 80% of the predicted 6MWT value (e.g., relative to a normal subject of about the same age, the same gender, and/or the same height) can be treated with an alkaline phosphatase or a polypeptide having alkaline

phosphatase activity (e.g. a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), such as by administering an alkaline phosphatase or a polypeptide having alkaline phosphatase activity for a treatment period of at least two weeks (e.g., at least three weeks, at least four weeks, at least five weeks, at least six weeks, at least seven weeks, at least eight weeks, at least nine weeks, at least ten weeks, at least three months, at least four months, at least five months, at least six months, at least seven months, at least eight months, at least nine months, at least one year, at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, or at least ten years, or the lifetime of the patient; particularly at least six weeks). For example, a subject having a muscle weakness disease with an average 6MWT of less than about 80% of the predicted 6MWT value (e.g., about 50%, about 55%, about 60%, about 65%, about 70%, or about 75% of the predicted 6MWT value) can be treated with an alkaline phosphatase or a polypeptide having alkaline phosphatase activity for a treatment period of at least two weeks (e.g., at least three weeks, at least four weeks, at least five weeks, at least six weeks, at least seven weeks, at least eight weeks, at least nine weeks, at least ten weeks, at least three months, at least four months, at least five months, at least six months, at least seven months, at least eight months, at least nine months, at least one year, at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, or at least ten years, or the lifetime of the patient; particularly at least six weeks).

The methods can result in an improvement in the 6MWT value of a subject having a muscle weakness disease. For example, treatment with an alkaline phosphatase or a polypeptide having alkaline phosphatase activity (e.g. a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), such as treatment with an alkaline phosphatase or a polypeptide having alkaline phosphatase activity for a treatment period of at least two weeks (e.g., at least three weeks, at least four weeks, at least five weeks, at least six weeks, at least seven weeks, at least eight weeks, at least nine weeks, at least ten weeks, at least three months, at least four months, at least five months, at least six months, at least seven months, at least eight months, at least nine months, at least one year, at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, or at least ten years, or the lifetime of the patient; particularly at least six weeks), can result in an average increase in the 6MWT value to about 80% or greater of the predicted 6MWT value of the patient (e.g. about 82%, about 84%, about 86%, about 88%, about 90%, about 92%, about 94%, about 96%, about 98%, or more of the predictive 6MWT value).

The increase in the 6MWT value of the subject having a muscle weakness disease can be sustained throughout administration of the alkaline phosphatase or a polypeptide having alkaline phosphatase activity (e.g. a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), e.g., for a treatment period of at least two weeks (e.g., at least three weeks, at least four weeks, at least five weeks, at least six weeks, at least seven weeks, at least eight weeks, at least nine weeks, at least ten weeks, at least three months, at least four months, at least five months, at least six

months, at least seven months, at least eight months, at least nine months, at least one year, at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, or at least ten years, or the lifetime of the subject; particularly at least six weeks). For instance, the 6MWT value increases to greater than about 80% of the predicted 6MWT value of the subject having a muscle weakness disease and remains at ± 10% of the increased 6MWT value during treatment with the alkaline phosphatase or a polypeptide having alkaline phosphatase activity.

Likewise, the improvement in walking ability of the subject having a muscle weakness disease can be sustained throughout administration of the alkaline phosphatase or a polypeptide having alkaline phosphatase activity, e.g., for a treatment period of at least two weeks (e.g., at least three weeks, at least four weeks, at least five weeks, at least six weeks, at least seven weeks, at least eight weeks, at least nine weeks, at least ten weeks, at least three months, at least four months, at least five months, at least six months, at least seven months, at least eight months, at least nine months, at least one year, at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, or at least ten years, or the lifetime of the patient; particularly at least six weeks). For instance, the subject having a muscle weakness disease exhibits decreased reliance on an assistive mobility device, such as a walker, a wheelchair, braces, crutches, or orthotics, during treatment with the sALP.

Alternatively, when administration of an alkaline phosphatase or a polypeptide having alkaline phosphatase activity (e.g. a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) does not result in an average increase in the 6MWT value to greater than 80% of the predicted 6MWT value (e.g., of a normal subject of about the same age, same gender, and/or height), the dosage and/or frequency of alkaline phosphatase or a polypeptide having alkaline phosphatase activity administration can be changed in order to determine the effective amount of the alkaline phosphatase or a polypeptide having alkaline phosphatase activity for the subject having a muscle weakness disease. For instance, the dosage of the alkaline phosphatase or a polypeptide having alkaline phosphatase activity can be increased from, e.g., about 2.1 mg/kg/week or about 3.5 mg/kg/week to about 6 mg/kg/week or about 9 mg/kg/week.

Handheld Dynamometry (HHD)

The grip and muscle strength of subjects having or being prone to a muscle weakness disease can be assessed using Hand Held Dynamometry (HHD). For example, knee flexion and extension and also hip flexion, extension, and abduction of a subject having or being prone to a muscle weakness disease can be measured using, e.g., a MICROFET2™ Dynamometer, while grip strength of the subject can be measured using, e.g., a Jamar Grip Dynamometer. In particular, the administrator holds the dynamometer stationary, and the subject exerts a maximal force against the dynamometer. Peak force data is collected in pounds, then converted to Newtons (N). Torque values are then calculated using limb length in N-meters. The torque value can then be compared to the torque value of, e.g., a normal subject

of about the same age, the same gender, and/or the same height, and expressed as a percentage value to generate the HHD value of the subject.

Subjects having a muscle weakness disease with an average HHD value of less than about 80% of the predicted HHD value (e.g., relative to a normal subject of about the same age, the same gender, and/or the same height) can be treated with an alkaline phosphatase or a polypeptide having alkaline phosphatase activity (e.g. a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), such as by administering an alkaline phosphatase or a polypeptide having alkaline phosphatase activity for a treatment period of at least two weeks (e.g., at least three weeks, at least four weeks, at least five weeks, at least six weeks, at least seven weeks, at least eight weeks, at least nine weeks, at least ten weeks, at least three months, at least four months, at least five months, at least six months, at least seven months, at least eight months, at least nine months, at least one year, at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, or at least ten years, or the lifetime of the patient; particularly at least six weeks). For example, a subject having a muscle weakness disease with an average HHD of less than about 80% of the predicted HHD value (e.g., about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, or about 75% of the predicted HHD value) can be treated with an alkaline phosphatase or a polypeptide having alkaline phosphatase activity for a treatment period of at least two weeks (e.g., at least three weeks, at least four weeks, at least five weeks, at least six weeks, at least seven weeks, at least eight weeks, at least nine weeks, at least ten weeks, at least three months, at least four months, at least five months, at least six months, at least seven months, at least eight months, at least nine months, at least one year, at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, or at least ten years, or the lifetime of the patient; particularly at least six weeks).

The methods can result in an improvement in the HHD value of a subject having a muscle weakness disease. For example, treatment with an alkaline phosphatase or a polypeptide having alkaline phosphatase activity (e.g. a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), such as treatment with an alkaline phosphatase or a polypeptide having alkaline phosphatase activity for a treatment period of at least two weeks (e.g., at least three weeks, at least four weeks, at least five weeks, at least six weeks, at least seven weeks, at least eight weeks, at least nine weeks, at least ten weeks, at least three months, at least four months, at least five months, at least six months, at least seven months, at least eight months, at least nine months, at least one year, at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, or at least ten years, or the lifetime of the patient; particularly at least six weeks), can result in an average increase in the HHD value to about 80% or greater of the predicted HHD value of the patient (e.g., about 83%, about 85%, about 87%, about 90%, about 93%, about 95%, about 97%, or about 100%, or about 100% of the predictive HHD value).

The increase in the HHD value of the subject having a muscle weakness disease can be sustained throughout administration of the alkaline phosphatase or a polypeptide having alkaline

phosphatase activity (e.g. a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), e.g., for a treatment period of at least two weeks (e.g., at least three weeks, at least four weeks, at least five weeks, at least six weeks, at least seven weeks, at least eight weeks, at least nine weeks, at least ten weeks, at least three months, at least four months, at least five months, at least six months, at least seven months, at least eight months, at least nine months, at least one year, at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, or at least ten years, or the lifetime of the subject; particularly at least six weeks). For instance, the HHD value increases to greater than about 80% of the predicted HHD value of the subject having a muscle weakness disease and remains at ± 10% of the increased HHD value during treatment with the alkaline phosphatase or a polypeptide having alkaline phosphatase activity.

Alternatively, when administration of an alkaline phosphatase or a polypeptide having alkaline phosphatase activity (e.g. a sALP, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) does not result in an average increase in the HHD value to greater than 80% of the predicted HHD value (e.g., of a subject having a muscle weakness disease of about the same age, same gender, and/or height), the dosage and/or frequency of alkaline phosphatase or a polypeptide having alkaline phosphatase activity administration can be changed in order to determine the effective amount of the alkaline phosphatase or a polypeptide having alkaline phosphatase activity for the subject having a muscle weakness disease. For instance, the dosage of the alkaline phosphatase or a polypeptide having alkaline phosphatase activity can be increased from, e.g., about 2.1 mg/kg/week or about 3.5 mg/kg/week to about 6 mg/kg/week or about 9 mg/kg/week.

Alkaline Phosphatase

Asfotase alfa is a human TNALP (hTNALP; SEQ ID NO: 1) fusion protein formulated for the treatment of HPP. In particular, asfotase alfa (SEQ ID NO: 1) can be used effectively to treat hypophosphatasia (HPP), its symptoms, and physical impairments associated therewith in a subject having or being prone to a muscle weakness disease for an extended period of time (e.g., at least one day, at least one week, at least two weeks, at least three weeks, at least one month, at least three months, at least six months, at least one year, at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or more than ten years (e.g., for the lifetime of the subject)).

Given the results described herein, the present disclosure is not limited to a particular alkaline phosphatase (ALP) or nucleic acid sequence encoding an ALP. Alkaline phosphatases encompass a group of enzymes that catalyze the cleavage of a phosphate moiety (e.g., hydrolysis of pyrophosphate, PP_i). There are four known mammalian alkaline phosphatase (ALP) isozymes: tissue nonspecific alkaline phosphatase (TNALP; described further below), placental alkaline phosphatase (PLALP) (e.g., Accession Nos. P05187, NP_112603, and NP_001623), germ cell alkaline phosphatase (GALP) (e.g., Accession No. P10696), and intestinal alkaline phosphatase (IALP) (e.g., Accession Nos. P09923 and NP_001622). In addition to the exemplary ALPs discussed above, this disclosure also provides any

polypeptide having the identical or similar catalytic site structure and/or enzymatic activity of ALP for treating subjects having or being prone to a muscle weakness disease. Bone delivery conjugates including sALP are further described in PCT publication Nos: WO 2005/103263 and WO 2008/138131.

TNALPs that can be used according to the methods described herein include, e.g., human TNALP (Accession Nos. NP_000469, AAI10910, AAH90861, AAH66116, AAH21289, and AAI26166); rhesus TNALP (Accession No. XP_01109717); rat TNALP (Accession No. NP_037191); dog TNALP (Accession No. AAF64516); pig TNALP (Accession No. AAN64273), mouse (Accession No. NP_031457), cow TNALP (Accession Nos. NP_789828, NP_776412, AAM 8209, and AAC33858), and cat TNALP (Accession No. NP_001036028). In particular, TNALP can be a recombinant human TNALP (e.g., SEQ ID NO: 1, asfotase alfa; see U.S. Patent Nos. 7,763,712 and 7,960,529, incorporated herein by reference in their entirety) used for the treatment of subjects having or being prone to a muscle weakness disease. The TNALP can also be one that exhibits at least about 95% sequence identity to the polypeptide or nucleic acid sequence of the above-noted TNALPs.

Soluble Alkaline Phosphatase

The ALPs of the present invention include soluble (e.g., extracellular or non-membrane-bound) forms of any of the alkaline phosphatases described herein. The sALP of the invention can be, for example, a soluble form of human tissue non-specific alkaline phosphatase (human TNALP (hTNALP)). The present disclosure is not limited to a particular sALP and can include any sALP polypeptide that is physiologically active toward, e.g., phosphoethanolamine (PEA), inorganic pyrophosphate (PPi), and pyridoxal 5'-phosphate (PLP). In particular, a sALP of the present invention is catalytically competent to improve skeletal mineralization in bone. The present disclosure further includes nucleic acids encoding the sALPs described herein that can be used to treat muscle weakness conditions described herein, including e.g., HPP, CPPD, familial hypophosphatemia (such as autosomal dominant hypophosphatemic rickets (ADHR), autosomal recessive hypophosphatemic rickets, X-linked hypophosphatemic rickets, X-linked hypophosphatemia (XLH), etc.), etc.

TNALP is a membrane-bound protein anchored by a glycolipid moiety at the C-terminal (Swiss-Prot, P05186). This glycolipid anchor (GPI) is added post-translationally after the removal of a hydrophobic C-terminal end, which serves both as a temporary membrane anchor and as a signal for the addition of the GPI. While the GPI anchor is located in the cell membrane, the remaining portions of TNALP are extracellular. In particular, TNALP (e.g., human TNALP (hTNALP)) can be engineered to replace the first amino acid of the hydrophobic C-terminal sequence (an alanine) with a stop codon, thereby producing an engineered hTNALP that contains all amino acid residues of the native anchored form of TNALP and lacks the GPI membrane anchor. One skilled in the art will appreciate that the position of the GPI membrane anchor will vary in different ALPs and can include, e.g., the last 10, 12, 14, 16, 18, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 34, 36, 38, 40, 45, 50, or more amino acid residues on the C-terminus of the polypeptide. Recombinant sTNALP can include, e.g., amino acids 1 to 502 (18 to 502 when secreted), amino acids 1 to 501 (18 to 501 when secreted), amino acids 1 to 504 (18 to 504

when secreted), amino acids 1 to 505 (18-505 when secreted), or amino acids 1 to 502. Thus, the C-terminal end of the native ALP can be truncated by certain amino acids without affecting ALP activity.

In addition to the C-terminal GPI anchor, TNALP also has an N-terminal signal peptide sequence. The N-terminal signal peptide is present on the synthesized protein when it is synthesized, but cleaved from TNALP after translocation into the ER. The sALPs of the invention include both secreted (i.e., lacking the N-terminal signal) and non-secreted (i.e., having the N-terminal signal) forms thereof. One skilled in the art will appreciate that the position of the N-terminal signal peptide will vary in different alkaline phosphatases and can include, for example, the first 5, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 30, or more amino acid residues on the N-terminus of the polypeptide. One of skill in the art can predict the position of a signal sequence cleavage site, e.g., by an appropriate computer algorithm such as that described in Bendtsen et al. (*J. Mol. Biol.* 340(4):783-795, 2004) and available on the Web at www.cbs.dtu.dk/services/SignalP/.

The present invention also includes sALP consensus sequences derived from the extracellular domain of ALP isozymes (e.g., TNALP, PALP, GCALP, IALP, etc.). Thus, similar to sTNALP discussed above, the present disclosure also provides other soluble human ALP isozymes, i.e., without the peptide signal, preferably comprising the extracellular domain of the ALPs. The sALPs of the invention also include polypeptide sequences satisfying a consensus sequence derived from the ALP extracellular domain of human ALP isozymes and of mammalian TNALP orthologs (human, mouse, rat, cow, cat, and dog) or a consensus derived from the ALP extracellular domain of just mammalian TNALP orthologs (human, mouse, rat, cow, cat, and dog). The sALPs of the invention also include those which satisfy similar consensus sequences derived from various combinations of these TNALP orthologs or human ALP isozymes. Such consensus sequences are given, for example, in WO 2008/138131.

sALPs of the present invention can include not only the wild-type sequence of the sALPs described above, but any polypeptide having at least 50% (e.g., 55%, 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to these alkaline phosphatases (e.g., SEQ ID NOs: 1-24; for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa). Examples of mutations that can be introduced into an ALP sequence are described in US Publication No. 2013/0323244, hereby incorporated by reference in its entirety. A sALP can optionally be glycosylated at any appropriate one or more amino acid residues. In addition, an sALP can have at least 50% (e.g., 55%, 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to any of the sALPs described herein (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa). A sALP can have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more additions, deletions, or substitutions relative to any of the sALPs described herein (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa).

sALP Fusion Polypeptides

Any of the sALPs and linkers described herein can be combined in a sALP polypeptide, e.g., a sALP polypeptide of A-sALP-B, wherein each of A and B is absent or is an amino acid sequence of at least one amino acid (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa). When present, A and/or B can be any linker described herein. In some sALP polypeptides, A is absent, B is absent, or A and B are both absent. The sALP polypeptides of the invention can optionally include an Fc region to provide an sALP fusion polypeptide, as described herein. The sALP polypeptide can optionally include a bone-targeting moiety, as described herein. In some sALP polypeptides, a linker, e.g., a flexible linker, can be included between the bone-targeting moiety and the sALP, such as a dipeptide sequence (e.g., leucine-lysine or aspartic acid-isoleucine). Further exemplary Fc regions, linkers, and bone-targeting moieties are described below.

Any of the sALPs, linkers, and Fc regions described herein can be combined in a fusion polypeptide, e.g., a recombinant fusion polypeptide, which includes the structure Z-sALP-Y-spacer-X-W_n-V, Z-W_n-X-spacer-Y-sALP-V, Z-sALP-Y-W_n-X-spacer-V, and Z-W_n-X-sALP-Y-spacer-V (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa). In particular, the structure can be Z-sALP-Y-spacer-X-W_n-V or Z-W_n-X-spacer-Y-sALP-V. The sALP can be the full-length or functional fragments of ALPs, such as the soluble, extracellular domain of the ALP, as is described herein (e.g., TNALP, PALP, GCALP and IALP). Any one of X, Y, Z, and V and/or the spacer can be absent or an amino acid sequence of at least one amino acid. W_n can be a bone-targeting moiety, e.g., having a series of consecutive Asp or Glu residues, in which n = 1 to 50, e.g., n = 3-30, e.g., 5-15, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 , 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 36, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50. The bone-targeting moiety, if present, can be positioned anywhere in the fusion polypeptide, e.g., at or near the N-terminal or C-terminal end, and/or in the linker region. For instance, the bone-targeting moiety is at the C-terminal end. sALP polypeptides and fusion polypeptides can also not include a bone-targeting moiety.

sALP fusion polypeptides of the present invention can be of the structure hTNALP-Fc-D_{10}. In particular, sALP fusion polypeptides can include an amino acid sequence of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa.

Useful spacers include, but are not limited to, polypeptides comprising an Fc, and hydrophilic and flexible polypeptides able to alleviate the repulsive forces caused by the presence of the terminal highly negatively charged peptide (e.g., W_n). For example, a sALP can be a fusion polypeptide including an Fc region of an immunoglobulin at the N-terminal or C-terminal domain. An immunoglobulin molecule has a structure that is well known in the art. It includes two light chains (~23 kD each) and two heavy chains (~50-70 kD each) joined by inter-chain disulfide bonds. Immunoglobulins are readily cleaved proteolytically (e.g., by papain cleavage) into Fab (containing the light chain and the VH and CH1 domains of the heavy chain) and Fc (containing the CH2 and CH3 domains of the heavy chain, along with adjoining sequences). Useful Fc fragments as described herein include the Fc fragment of any

immunoglobulin molecule, including IgG, IgM, IgA, IgD, or IgE, and their various subclasses (e.g., IgG-1, IgG-2, IgG-3, IgG-4, IgA-1, IgA-2), from any mammal (e.g., human). For instance, the Fc fragment is human IgG-1. The Fc fragments of the invention can include, for example, the CH2 and CH3 domains of the heavy chain and any portion of the hinge region. The Fc region can optionally be glycosylated at any appropriate one or more amino acid residues known to those skilled in the art. In particular, the Fc fragment of the fusion polypeptide has the amino acid sequence of SEQ ID NO: 20, or has at least 50% (e.g., 55%, 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO: 20. Engineered, e.g., non-naturally occurring, Fc regions can be utilized in the methods of the invention, e.g., as described in International Application Pub. No. WO2005/007809, which is hereby incorporated by reference. An Fc fragment as described herein can have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 50, or more additions, deletions, or substitutions relative to any of the Fc fragments described herein.

The sALP fusion polypeptides described herein (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can include a peptide linker region between the Fc fragment. In addition, a peptide linker region can be included between the Fc fragment and the optional bone-targeting moiety. The linker region can be of any sequence and length that allows the sALP to remain biologically active, e.g., not sterically hindered. Exemplary linker lengths are between 1 and 200 amino acid residues, e.g., 1-5, 6-10, 11-15, 16-20, 21-25, 26-30, 31-35, 36-40, 41-45, 46-50, 51-55, 56-60, 61-65, 66-70, 71-75, 76-80, 81-85, 86-90, 91-95, 96-100, 101-110, 111-120, 121-130, 131-140, 141-150, 151-160, 161-170, 171-180, 181-190, or 191-200 amino acid residues. For instance, linkers include or consist of flexible portions, e.g., regions without significant fixed secondary or tertiary structure. Exemplary flexible linkers are glycine-rich linkers, e.g., containing at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or even 100% glycine residues. Linkers can also contain, e.g., serine residues. In some cases, the amino acid sequence of linkers consists only of glycine and serine residues. A linker can optionally be glycosylated at any appropriate one or more amino acid residues. Additionally, a linker as described herein can include any other sequence or moiety, attached covalently or non-covalently. The linker can also be absent, in which the Fc fragment and the sALP are fused together directly, with no intervening residues. Certain Fc-sALP or sALP-Fc fusion polypeptides can be viewed, according to the present disclosure, either as 1) having no linker, or as 2) having a linker which corresponds to a portion of the sALP. For example, Fc fused directly to hsTNALP (1-502) can be viewed, e.g., either as having no linker, in which the hsTNALP is amino acids 1-502, or as having a 17-amino acid linker, in which the hsTNALP (18-502).

Additional amino acid residues can be introduced into the polypeptide according to the cloning strategy used to produce the fusion polypeptides. For instance, the additional amino acid residues do not provide an additional GPI anchoring signal so as to maintain the polypeptide in a soluble form. Furthermore, any such additional amino acid residues, when incorporated into the polypeptide of the invention, do not provide a cleavage site for endoproteases of the host cell. The likelihood that a

designed sequence would be cleaved by the endoproteases of the host cell can be predicted as described, e.g., by Ikezawa (*Biol. Pharm. Bull.* 25:409-417, 2002).

The sALPs and sALP fusion polypeptides of the invention (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be associated into dimers or tetramers. For example, two sALP-Fc monomers can covalently be linked through two disulfide bonds located in the hinge regions of the Fc fragments. Additionally, the polypeptide or fusion polypeptide of the invention (e.g., a sALP polypeptide or fusion polypeptide) can be glycosylated or PEGylated.

Production of Nucleic Acids and Polypeptides

The nucleic acids encoding sALPs and sALP fusion polypeptides of the invention (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be produced by any method known in the art. Typically, a nucleic acid encoding the desired fusion polypeptide is generated using molecular cloning methods, and is generally placed within a vector, such as a plasmid or virus. The vector is used to transform the nucleic acid into a host cell appropriate for the expression of the fusion polypeptide. Representative methods are disclosed, for example, in Maniatis et al. (Cold Springs Harbor Laboratory, 1989). Many cell types can be used as appropriate host cells, although mammalian cells are preferable because they are able to confer appropriate post-translational modifications. Host cells of the present invention can include, e.g., Chinese Hamster Ovary (CHO) cell, L cell, C127 cell, 3T3 cell, BHK cell, COS-7 cell or any other suitable host cell known in the art. For example, the host cell is a Chinese Hamster Ovary (CHO) cell (e.g., a CHO-DG44 cell).

The sALPs and sALP fusion polypeptides (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be produced under any conditions suitable to effect expression of the sALP polypeptide in the host cell. Such conditions include appropriate selection of a media prepared with components such as a buffer, bicarbonate and/or HEPES, ions like chloride, phosphate, calcium, sodium, potassium, magnesium, iron, carbon sources like simple sugars, amino acids, potentially lipids, nucleotides, vitamins and growth factors like insulin; regular commercially available media like alpha-MEM, DMEM, Ham's-F12, and IMDM supplemented with 2-4 mM L-glutamine and 5% Fetal bovine serum; regular commercially available animal protein free media like Hyclone™ SFM4CHO, Sigma CHO DHFR$^-$, Cambrex POWER™ CHO CD supplemented with 2-4 mM L-glutamine. These media are desirably prepared without thymidine, hypoxanthine and L-glycine to maintain selective pressure, allowing stable protein-product expression.

Pharmaceutical compositions and formulations

A composition of the present invention (e.g., including a sALP or sALP fusion polypeptide, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be administered by a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. The route of administration can depend on a variety of factors, such as the environment and therapeutic goals. In particular, the polypeptides and fusion polypeptides described herein can be administration by any route known in the art, e.g., subcutaneous (e.g., by subcutaneous injection), intravenously, orally, nasally, intramuscularly, sublingually, intrathecally, or intradermally. By way of example, pharmaceutical compositions of the invention can be in the form of a liquid, solution, suspension, pill, capsule, tablet, gelcap, powder, gel, ointment, cream, nebulae, mist, atomized vapor, aerosol, or phytosome.

Dosage

Any amount of a pharmaceutical composition (e.g., including a sALP or sALP fusion polypeptide, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be administered to a subject having or being prone to a muscle weakness disease. The dosages will depend on many factors including the mode of administration and the age of the patient. Typically, the amount of the composition (e.g., a sALP or sALP fusion polypeptide, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) contained within a single dose will be an amount that is effective to treat a condition (e.g., HPP) as described herein without inducing significant toxicity.

For example, the sALP polypeptides (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) described herein can be administered to an subject having or being prone to a muscle weakness disease, in individual doses ranging, e.g., from 0.01 mg/kg to 500 mg/kg (e.g., from 0.05 mg/kg to 500 mg/kg, from 0.1 mg/kg to 20 mg/kg, from 5 mg/kg to 500 mg/kg, from 0.1 mg/kg to 100 mg/kg, from 10 mg/kg to 100 mg/kg, from 0.1 mg/kg to 50 mg/kg, 0.5 mg/kg to 25 mg/kg, 1.0 mg/kg to 10 mg/kg, 1.5 mg/kg to 5 mg/kg, or 2.0 mg/kg to 3.0 mg/kg) or from 1 µg/kg to 1,000 µg/kg (e.g., from 5 µg/kg to 1,000 µg/kg, from 1 µg/kg to 750 µg/kg, from 5 µg/kg to 750 µg/kg, from 10 µg/kg to 750 µg/kg, from 1 µg/kg to 500 µg/kg, from 5 µg/kg to 500 µg/kg, from 10 µg/kg to 500 µg/kg, from 1 µg/kg to 100 µg/kg, from 5 µg/kg to 100 µg/kg, from 10 µg/kg to 100 µg/kg, from 1 µg/kg to 50 µg/kg, from 5 µg/kg to 50 µg/kg, or from 10 µg/kg to 50 µg/kg).

Exemplary doses of a sALP include, e.g., 0.01, 0.05, 0.1, 0.5, 1, 2, 2.5, 5, 10, 20, 25, 50, 100, 125, 150, 200, 250, or 500 mg/kg; or 1, 2, 2.5, 5, 10, 20, 25, 50, 100, 125, 150, 200, 250, 500, 750, 900, or 1,000 µg/kg. For all dosages or ranges recited herein, the term "about" can be used to modify these dosages by ±10% of the recited values or range endpoints. In particular, compositions (e.g., including sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa)) in

accordance with the present disclosure can be administered to patients in doses ranging from about 0.001 mg/kg/day to about 500 mg/kg/day, about 0.01 mg/kg/day to about 100 mg/kg/day, or about 0.01 mg/kg/day to about 20 mg/kg/day. For example, the sALP compositions (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be administered to patients in a weekly dosage ranging, e.g., from about 0.5 mg/kg/week to about 140 mg/kg/week, e.g., about 0.8 mg/kg/week to about 50 mg/kg/week, or about 1 mg/kg/week to about 10 mg/kg/week (e.g., about 6 or about 9 mg/kg/week). In particular, the sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be administered at a dosage of 2 mg/kg three times a week (total dose 6 mg/kg/week), 1 mg/kg six times a week (total dose 6 mg/kg/week), 3 mg/kg three times a week (total dose 9 mg/kg/week), 0.5 mg/kg three times a week (total dose of 1.5 mg/kg/week), or 9.3 mg/kg three times a week (total dose 28 mg/kg/week). The dosage will be adapted by the clinician in accordance with conventional factors such as the extent of the disease and different parameters from the subject having or being prone to a muscle weakness disease.

Dosages of compositions including sALPs and sALP fusion polypeptides (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be provided in either a single or multiple dosage regimens. Doses can be administered, e.g., hourly, bi-hourly, daily, bi-daily, twice a week, three times a week, four times a week, five times a week, six times a week, weekly, biweekly, monthly, bimonthly, or yearly. Alternatively, doses can be administered, e.g., twice, three times, four times, five times, six times, seven times, eight times, nine times, 10 times, 11 times, or 12 times per day. In particular, the dosing regimen is once weekly. The duration of the dosing regimen can be, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 day(s), week(s), or month(s), or even for the remaining lifespan of the subject having or being prone to a muscle weakness disease. The amount, frequency, and duration of dosage will be adapted by the clinician in accordance with conventional factors such as the extent of the disease and different parameters from the subject having or being prone to a muscle weakness disease.

For example, a sALP or sALP fusion polypeptide (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be formulated as a solution for injection, which is a clear, colorless to slightly yellow, aqueous solution, pH 7.4. The sALP or sALP polypeptide (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) may be formulated at a concentration of 12mg/0.3mL,18mg/0.45mL, 28mg/0.7mL, 40mg/1ml, or 80mg/0.8mL. In particular, the composition can be formulated as a 40 mg/ml solution for injection, in which each ml of solution contains 40 mg of sALP or sALP polypeptide (e.g., each vial contains 0.3 ml solution and 12 mg of sALP (40 mg/ml), each vial contains 0.45 ml solution and 18 mg of sALP (40 mg/ml), each vial contains 0.7 ml solution and 28 mg of sALP(40 mg/ml), or each vial contains 1.0 ml solution and 40 mg of asfotase alfa (40 mg/ml)). A sALP or sALP polypeptide (such as TNALP, for example the sALP polypeptide of SEQ ID

NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be formulated as a solution for injection at a concentration of 100 mg/ml, in which each 1 ml of solution contains 100 mg of sALP or sALP polypeptide (e.g., each vial contains 0.8 ml solution and 80 mg of asfotase alfa (100 mg/ml)).

For example, the recommended dosage of a sALP or sALP fusion polypeptide ((such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) is 2 mg/kg of body weight administered subcutaneously three times per week, or a dosage regimen of 1 mg/kg of body weight administered subcutaneously six times per week. Additional dosage information is provided below (Table 1).

Table 1. DOSING OF ASFOTASE ALFA

Body Weight (kg)	If injecting 3x per week			If injecting 6 x per week		
	Dose to be injected	Volume to be injected	Vial type used for injection	Dose to be injected	Volume to be injected	Vial type used for injection
3	6 mg	0.15 ml	0.3 ml			
4	8 mg	0.20 ml	0.3 ml			
5	10 mg	0.25 ml	0.3 ml			
6	12 mg	0.30 ml	0.3 ml	6 mg	0.15 ml	0.3 ml
7	14 mg	0.35 ml	0.45 ml	7 mg	0.18 ml	0.3 ml
8	16 mg	0.40 ml	0.45 ml	8 mg	0.20 ml	0.3 ml
9	18 mg	0.45 ml	0.45 ml	9 mg	0.23 ml	0.3 ml
10	20 mg	0.50 ml	0.7 ml	10 mg	0.25 ml	0.3 ml
11	22 mg	0.55 ml	0.7 ml	11 mg	0.28 ml	0.3 ml
12	24 mg	0.60 ml	0.7 ml	12 mg	0.30 ml	0.3 ml
13	26 mg	0.65 ml	0.7 ml	13 mg	0.33 ml	0.45 ml
14	28 mg	0.70 ml	0.7 ml	14 mg	0.35 ml	0.45 ml
15	30 mg	0.75 ml	1 ml	15 mg	0.38 ml	0.45 ml
16	32 mg	0.80 ml	1 ml	16 mg	0.40 ml	0.45 ml
17	34 mg	0.85 ml	1 ml	17 mg	0.43 ml	0.45 ml
18	36 mg	0.90 ml	1 ml	18 mg	0.45 ml	0.45 ml
19	38 mg	0.95 ml	1 ml	19 mg	0.48 ml	0.7 ml
20	40 mg	1.00 ml	1 ml	20 mg	0.50 ml	0.7 ml
25	50 mg	0.50 ml	0.8 ml	25 mg	0.63 ml	0.7 ml
30	60 mg	0.60 ml	0.8 ml	30 mg	0.75 ml	1 ml
35	70 mg	0.70 ml	0.8 ml	35 mg	0.88 ml	1 ml
40	80 mg	0.80 ml	0.8 ml	40 mg	1.00 ml	1 ml
50				50 mg	0.50 ml	0.8 ml
60				60 mg	0.60 ml	0.8 ml
70				70 mg	0.70 ml	0.8 ml
80				80 mg	0.80 ml	0.8 ml
90				90 mg	0.90 ml	0.8 ml (x2)
100				100 mg	1.00 ml	0.8 ml (x2)

Formulations

The compositions including sALPs and sALP fusion polypeptides (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be formulated according to standard methods. Pharmaceutical formulation is a well-established art, and is further described in, e.g., Gennaro (2000) "Remington: The Science and Practice of Pharmacy," 20th Edition, Lippincott, Williams & Wilkins (ISBN: 0683306472); Ansel et al. (1999) "Pharmaceutical Dosage Forms and Drug Delivery Systems," 7th Edition, Lippincott Williams & Wilkins Publishers (ISBN: 0683305727); and Kibbe (2000) "Handbook of Pharmaceutical Excipients American Pharmaceutical Association," 3rd Edition (ISBN: 091733096X). For instance, a sALP composition (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be formulated, for example, as a buffered solution at a suitable concentration and suitable for storage at 2-8°C (e.g., 4°C). A composition can also be formulated for storage at a temperature below 0°C (e.g., -20°C or -80°C). A composition can further be formulated for storage for up to 2 years (e.g., one month, two months, three months, four months, five months, six months, seven months, eight months, nine months, 10 months, 11 months, 1 year, 1½ years, or 2 years) at 2-8°C (e.g., 4°C). Thus, the compositions described herein can be stable in storage for at least 1 year at 2-8°C (e.g., 4°C).

The compositions including sALPs and sALP fusion polypeptides (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be in a variety of forms. These forms include, e.g., liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends, in part, on the intended mode of administration and therapeutic application.

For example, compositions intended for systemic or local delivery can be in the form of injectable or infusible solutions. Accordingly, the compositions (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be formulated for administration by a parenteral mode (e.g., subcutaneous, intravenous, intraperitoneal, or intramuscular injection). "Parenteral administration," "administered parenterally," and other grammatically equivalent phrases, as used herein, refer to modes of administration other than enteral and topical administration, usually by injection, and include, without limitation, subcutaneous, intradermal, intravenous, intranasal, intraocular, pulmonary, intramuscular, intra-arterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intrapulmonary, intraperitoneal, transtracheal, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural, intracerebral, intracranial, intracarotid, and intrasternal injection and infusion.

The compositions including sALPs and sALP fusion polypeptides (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable for stable storage at high concentration. Sterile injectable solutions can be prepared by incorporating a composition described herein in the required

amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filter sterilization. Generally, dispersions are prepared by incorporating a composition described herein into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods for preparation include vacuum drying and freeze-drying that yield a powder of a composition described herein plus any additional desired ingredient (see below) from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition a reagent that delays absorption, for example, monostearate salts, and gelatin.

The compositions described herein can also be formulated in immunoliposome compositions. Such formulations can be prepared by methods known in the art such as, e.g., the methods described in Epstein et al. (1985) *Proc Natl Acad Sci USA* 82:3688; Hwang et al. (1980) *Proc Natl Acad Sci USA* 77:4030; and U.S. Patent Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in, e.g., U.S. Patent No. 5,013,556.

Compositions including sALPs and sALP fusion polypeptides (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can also be formulated with a carrier that will protect the composition (e.g., a sALP polypeptide or sALP fusion polypeptide) against rapid release, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are known in the art. See, e.g., J.R. Robinson (1978) "Sustained and Controlled Release Drug Delivery Systems," Marcel Dekker, Inc., New York.

When compositions are to be used in combination with a second active agent, the compositions can be co-formulated with the second agent, or the compositions can be formulated separately from the second agent formulation. For example, the respective pharmaceutical compositions can be mixed, e.g., just prior to administration, and administered together or can be administered separately, e.g., at the same or different times.

Compositions including sALPs and sALP fusion polypeptides (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be formulated for administration to a patient or, if administered to a fetus, to a female carrying such fetus, along with intravenous gamma globulin therapy (IVIG), plasmapheresis, plasma replacement, or plasma exchange.

Carriers/vehicles

Preparations containing a sALP or sALP fusion polypeptide (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be provided to subjects having or being prone to a

muscle weakness disease, in combination with pharmaceutically acceptable sterile aqueous or non-aqueous solvents, suspensions or emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil, fish oil, and injectable organic esters. Aqueous carriers include water, water-alcohol solutions, emulsions or suspensions, including saline and buffered medical parenteral vehicles including sodium chloride solution, Ringer's dextrose solution, dextrose plus sodium chloride solution, Ringer's solution containing lactose, or fixed oils. For example, the pharmaceutically acceptable carrier can include sodium chloride and/or sodium phosphate, in which the composition includes, e.g., about 150 mM sodium chloride and/or about 25 mM sodium phosphate, pH 7.4.

Intravenous vehicles can include fluid and nutrient replenishers, electrolyte replenishers, such as those based upon Ringer's dextrose, and the like. Pharmaceutically acceptable salts can be included therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, can be present in such vehicles. A thorough discussion of pharmaceutically acceptable carriers is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

The following examples are intended to illustrate, rather than limit, the disclosure.

EXAMPLES

Example 1. AKP2$^{-/-}$ Mice Study

AKP2$^{-/-}$ knockout mice are created by inactivating the gene *AKP2*, which encodes the mouse counterpart of human tissue-non-specific alkaline phosphatase (TNSALP). *AKP2*$^{-/-}$ knockout mice have been used as a model of human HPP that recapitulates HPP with onset in infancy (Narisawa et al. *Dev Dyn.* 1997; 208:432-446). This Example summarizes the effects to examine muscle fiber composition and strength in *AKP2*$^{-/-}$ mice compared with wild type (WT) mice, and to determine the effect of asfotase alfa in correcting the muscle weakness phenotype in the *AKP2*$^{-/-}$ mice.

Specifically, the soleus and the extensor digitorum longus (EDL) muscles were examined. To determine the muscle fiber composition, the fiber size and type were measured or detected. Muscles were harvested, sectioned and subjected to immunohistochemistry with antibodies recognizing laminin or myosin heavy chain I, IIa, or IIB, as described previously (see Barton et al. 2005 *J Orthop Res.* 23: 259-265; Barton et al. 2012 *Faseb J.* 26: 3691-3702; and Evans et al. 2008 *Physiol. Genomics* 35: 86-95). Image acquisition was performed on a Leica DMR epifluorescence microscope using OpenLab software. Fiber size and type was determined using MatLAB, where the laminin signal defined the boundary of each muscle fiber, and the anti-myosin antibodies detected the fiber type.

To determine the muscle fiber strength, contractile properties of soleus and EDL muscles of mice were monitored about 2 weeks from birth. Properties to be measured include: maximum force generating capacity using 120 and 100 Hz at supramaximal stimulation current; specific force (force per cross-sectional area; force frequency via calcium handling and/or fiber type differences; and fatigue using 330 msec stimulation duration every 1 sec (33% duty ratio). Effect of PPi level on the contractile function of soleus and EDL muscles was measured by exposing the dissected muscles to a range of PPi

concentrations (e.g., 2, 4, 8, and 10 μM). In one exemplary experiment, Group 1 muscles were exposed to 1 and 8 μM or 4 and 10 μM and Group 2 muscles were exposed to 4 and 8 μM or 2 and 10 μM.

As the result, no difference on fiber type proportion was observed between the soleus muscles from *AKP2*$^{-/-}$ mice and the soleus muscles from wild type (WT) mice (Fig. 1). Compared to muscles from wild type (WT) mice, the *AKP2*$^{-/-}$ muscles had smaller fibers over all (Fig. 2). For example, the *AKP2*$^{-/-}$ muscle had more percentages of type 1 fibers (Fig. 2B), type IIa fibers (Fig. 2C), and type IIb fibers (Fig. 2D) of short size (e.g., less than 260 μm^2). Interestingly, the *AKP2*$^{-/-}$ muscle had a small population of larger myosin IIb fibers, while the wild type muscles lacked such fibers (Fig. 2D). One factor that may account for this is the small proportion of IIb fibers within the soleus muscle (~2%). Mature soleus muscles rarely have IIb fibers, but immature muscles have a faster muscle phenotype than mature muscles, and so the residual IIb fibers are still evident at this age. Taken together, the *Akp2*$^{-/-}$ mouse had smaller fibers with no apparent shift in fiber type.

Isolated muscle function testing was performed on the EDL and Soleus muscles from 2 week old *AKP2*$^{-/-}$ mice and WT controls. Both males and females were tested to distinguish any differences in force generation dependent upon gender.

Maximum force generating capacity was tested using 120 and 100 Hz for EDL and soleus at supramaximal stimulation current. Specific force (Force per cross sectional area) was determined for all muscles. There was no statistical difference in strength between different strains or different genders. Force frequency relationships were also determined as an assessment of calcium handling and/or fiber-type differences. There were no apparent differences between groups. Fatigue tests were also performed, using a 330 msec stimulation duration every 1 sec (33% duty ratio). There were no apparent differences between groups. As shown in Figure 3, no difference was observed between the soleus muscles from *AKP2*$^{-/-}$ mice and the soleus muscles from wild type (WT) mice for the mass (Fig. 3A), strength (Fig. 3B), force frequency (Fig. 3C), or fatigue parameters (Fig. 3D). Similarly, no difference was observed between the EDL muscles from *AKP2*$^{-/-}$ mice and the EDL muscles from wild type (WT) mice for the mass (Fig. 4A), strength (Fig. 4B), force frequency (Fig. 4C), or fatigue parameters (Fig. 4D). Note that for the figures of frequency and fatigue, error bars were not displayed for clarity. Although the N was low (N=3) for the fatigue and force-frequency results, the lack of any overt differences between groups suggests that there was truly no difference. This result is consistent with the fiber type distributions measured previously (Fig. 2).

HPP patients, CPPD patients, and the *Akp2*$^{-/-}$ mice all have elevated PPi in their circulation. Thus, it is likely that these elevated levels equilibrate with the muscles. The effects of PPi concentration on muscle contractile functions were then tested. A pilot study was performed using 10 μM PPi, while the wild type (WT) muscles exhibited a reversible loss in force production when exposed to 10 μM PPi. The effect of high PPi on force in muscles from WT and *Akp2*$^{-/-}$ mice was then tested on a range of concentrations were tested (2, 4, 8, 10 μM) to bracket the physiological level found in the *Akp2*$^{-/-}$ mice and in HPP patients. An initial cohort of muscles was tested for 2 and 8 μM, or 4 and 10 μM PPi concentration. A second cohort of muscles was tested at 4 and 8 or 2 and 10 μM. Muscles were first tested in normal Ringers solution, followed by the two test conditions for 30 minutes each, and ending with a return to normal Ringers. Data from muscles that did not return to the initial force values in normal

Ringers was discarded. As shown in Figure 5B, EDL muscles from *AKP2*$^{-/-}$ mice were more sensitive to elevated PPi than EDL muscles from wild type (WT) mice. For example, more than 4 μM PPi reduced the relative force of EDL muscles from *AKP2*$^{-/-}$ mice, while the relative force of EDL muscles from wild type (WT) mice did not change dramatically unless the PPi concentration was at least 10 μM (Fig. 5B). On the contrary, the soleus muscles from *AKP2*$^{-/-}$ mice and wild type (WT) mice were sensitive to elevated PPi in a similar degree (Fig. 5A).

Future experiments to explore the effects of PPi could be performed. For instance, in elevated PPi, the force-frequency or fatigability could be altered, exacerbating weakness. If this is to be pursued, using a single PPi concentration (e.g., 8 μM) would simplify the study.

Asfotase alfa was then administered to *AKP2*$^{-/-}$ mice to determine if there is a correlation between the decreased muscle force and the increased PPi circulating levels in *AKP2*$^{-/-}$ mice and to evaluate the asfotase alfa efficacy in correcting the related phenotype. Since untreated *AKP2*$^{-/-}$ mice typically die at about 12 days of age (the life span may be extended to 18-20 days, if supplemented with pyridoxine, but it is still not sufficient for muscle measurements) and are too young to measure muscle force *in vivo*, there was a difficulty to use untreated *AKP2*$^{-/-}$ mice as a control to analyze the treatment effect of asfotase alfa. Instead, a withdrawal experiment was used. Specifically, *AKP2*$^{-/-}$ mice were treated with asfotase alfa from birth until 35 days of age. At that time, some mice were withdrawn from treatment, and their PPi concentration and muscle force were measured and compared to those of mice receiving continued treatment. The whole study design is summarized as below:

Table 2. Open Label Treatment with Parallel and Randomized Control Study Design.

Group Number	Group Description	Test Article	Route	Treatment Duration with asfotase alfa (Day)	Treatment duration with vehicle (Day)	Dosing interval	Dose level (mg/kg)	N= Per bleeding and grip force day (i.e., Day 36, 39, 42)
1	Homo_Tx-V	35 days with asfotase alfa followed by 3 or 6 days with Vehicle	SC injection	35 (Day 1 to Day 35)	3 or 6 (Day 36 to Day 38 or 41)	Once a day	8.2	12 *Akp2*$^{-/-}$ (Total: 24*) *Day 36 will not be performed (equivalent to group 2 at day 36)
2	Homo_Tx-Tx	asfotase alfa	SC injection	35, 38, or 41 (Day 1 to Day 35, 38, or 41)	---	Once a day	8.2	12 *Akp2*$^{-/-}$ (Total: 36)
3	WT	---	---	---	---	---	---	12 WT (Total: 36)

4	WT_Tx-V	35 days with asfotase alfa followed by 0, 3, or 6 days with Vehicle	SC injection	35 (Day 1 to Day 35)	0, 3, or 6 (None or Day 36 to Day 38 or 41)	Once a day	8.2	12 WT (Total: 36)

WT: represents wild-type littermate of *Akp2*$^{-/-}$ mice

8.2 mg/kg asfotase alfa was subcutaneously (SC) administered to *AKP2*$^{-/-}$ mice once daily from the day of birth to Day 35 after birth. Half of knockout mice then continued receiving subcutaneous administration of asfotase alfa in the same dosage regimen, while the other half received subcutaneous administration of the control vehicle in the same dosage regimen. On Day 42 both groups of *AKP2*$^{-/-}$ mice, as well as untreated wild type (WT) C57BL/6-129J mice, were tested for their grip force.

Five trials were performed. Scores were averaged among these trials and normalized to body weight. The grip strength of forelimbs and hindlimbs were measured and compared among wild type mice (WT), *AKP2*$^{-/-}$ mice receiving continuous treatment (Tx-Tx), and *AKP2*$^{-/-}$ mice with discontinued treatment after Day 35 (Tx-V). *AKP2*$^{-/-}$ mice receiving continuous treatment (Tx-Tx) showed stronger grip strength, for both fore- and hindlimbs, compared to *AKP2*$^{-/-}$ mice with discontinued treatment after Day 35 (Tx-V), demonstrating the beneficial effect of continuous asfotase alfa treatment on a muscle weakness disease (Fig. 6).

These mice studies suggest that muscle weakness (observed in HPP) is present in the mouse model. More surprisingly, they suggest that muscle weakness in HPP is probably not due to the bone defect (which is taken as the characteristic feature of HPP), since no difference among wild type (WT) mice and *AKP2*$^{-/-}$ mice were observed in their soleus fiber type proportions or soleus or EDL muscle contractile properties ex vivo, even *AKP2*$^{-/-}$ mice had some degree of smaller muscle fibers. On the contrary, muscle weakness in HPP was more correlated to the elevated PPi concentration, since reducing PPi by administering asfotase alfa improved *AKP2*$^{-/-}$ mice muscle grip strength. Thus, a human patient having a muscle weakness disease characterized by elevated PPi concentration, even without other HPP symptoms or not being diagnosed with HPP yet, may still be treated by asfotase alfa. Similarly, patients having or being prone to other muscle weakness diseases, such as CPPD patients and familial hypophosphatemia patients, may also be treated by decreasing elevated PPi or other alkaline phosphatase substrates (e.g., PLP, PEA, etc.) by administration of asfotase alfa.

Example 2. Treating muscle weakness in humans

As illustrated in studies in *AKP2*$^{-/-}$ knockout mice described above, there is a correlation between elevated PPi circulating levels (due to decreased alkaline phosphatase activity) and decreased muscle force. Such correlation may also exist in HPP patients. Asfotase alfa treatment may also be effective to correct the muscle weakness phenotype of HPP patients, or patients with other muscle weakness diseases characterized with low alkaline phosphatase activity and/or elevated PPi concentration (such as in CPPD and/or familial hypophosphatemia). This Example discloses methods of identifying a subpopulation of patients having a muscle weakness disease (e.g., HPP, CPPD, familial

hypophosphatemia, etc.) with low alkaline phosphatase activity and/or elevated PPi concentration, and methods of treating, or ameliorating the muscle weakness phenotype of, a patient in such subpopulation with asfotase alfa. A patient may be identified as one of such subpopulation if having an increased PPi concentration (or an increased concentration of at least one alkaline phosphatase substrate, such as PLP and PEA) and a muscle weakness phenotype (e.g., loss of muscle force). Additionally, the patient may be identified as having a low alkaline phosphatase concentration (Table 4).

Table 3. Low and normal alkaline phosphatase concentrations in females and males by age group.

Age	Female		Male	
	Low ALP (U/L)	Normal ALP (U/L)	Low ALP (U/L)	Normal ALP (U/L)
0 - 14 d	90	273	90	273
15 d - < 1 y	134	518	134	518
1 - < 10 y	156	369	156	369
10 - < 13 y	141	460	141	460
13 - < 15 y	62	280	127	517
15 - < 17 y	54	128	89	365
17 - < 19 y	48	95	59	164

Identification of the muscle weakness disease or phenotype may be done using routine technologies known in the art. Measurement of PPi (or PLP, PEA, or other alkaline phosphatase substrates) concentration in such patient may also be carried out using routine technologies known in the art and be compared to the PPi concentration of normal subjects or subjects without such muscle weakness disease or phenotype (Table 3). Elevated PPi concentration may then be identified through this comparison.

Table 4. Normal ranges of pyrophosphate (PPi) levels in infants and children, adolescents, and adults.

Category	Age	Samples(N)	Range (µM)
Infants & Children	< 12	100	1.33 – 5.71
Adolescents	13-18	120	<0.75 – 4.78
Adult	> 18	120	1.00 – 5.82

More commonly, the level of alkaline phosphatase activity in serum or plasma is measured and compared to age and sex adjusted normative data. The *AKP2*$^{-/-}$ knockout mice studies were performed to the murine soleus and the murine EDL muscles in order to understand the underlying etiology for hypotonia, which would be considered to be an excessively invasive test if performed in humans. Additionally, because the murine muscle tissue is physiologically plastic, data from murine muscle would be expected to be less affected than the corresponding human muscle tissue. Therefore, small changes in murine response would correlate to a larger response in human muscle tissue. In addition, because PPi is not a commercially available assay, alkaline phosphatase activity is the accepted surrogate marker for PPi levels (and are inversely correlated).

The same or different muscles may be tested for diagnosing muscle weakness diseases or

phenotypes in animals or humans. For example, other skeletal or striated muscles, or cardiac or smooth muscles may be tested for various properties. For example, the passive mechanical properties (e.g., the Calcaneus Segment properties) of muscles (e.g., the gastrocnemius muscle and the Achilles Tendon) may be tested with methods known in the art. The viscoelastic property of muscle stiffness may also be tested.

Asfotase alfa was previously shown effective to treat HPP patients and a dosage of 3, 6, or 9 mg/kg/week was suggested for subcutaneous administration three times per week or once per day. The same dosage regimens, or a different one after similar studies as illustrated in Example 1, may be given to HPP patients, CPPD patients, or hypophosphatemia patients without HPP to treat the muscle weakness phenotype. To test the treatment effect, multiple endpoints may be used. Some such endpoints used in HPP treatment include, for example, the Bruininks-Oseretsky Test of Motor Proficiency 2nd Edition (BOT-2), the Radiographic Global Impression of Change (RGI-C) scale (a 7-point scale in which a rating of -3 represents severe worsening and a rating of +3 indicates near or complete healing), the Bayley Scales of Infant and Toddler Development, 3rd Edition (BSID-III), the Childhood Health Assessment Questionnaire (CHAQ), the Pediatric Outcomes Data Collection Instrument test (PODCI), the Peabody Developmental Motor Scales, 2nd Edition (PDMS-2), six-minute walk test (6MWT), the 12-point performance-oriented mobility assessment (POMA-G), a modified performance-oriented mobility assessment (mPOMA-G, such as the one illustrated in Phillips et al. 2015 *Bone Abstracts* 4:P136), and other methods or tests known in the art. Both naïve patients and patients having been administered with other alkaline phosphatase therapy may be treated with asfotase alfa or other related polypeptides having alkaline phosphatase activity.

Example 3. Treating muscle weakness in patient 1

A patient was identified as having hypotonia in conjunction with low ALP (correlated with high PPi), elevated PLP, and elevated urinary PEA. A 6-year-old patient presented with hypotonia of unknown etiology. The patient's additional conditions included cerebellar atrophy, axonal sensory and motor peripheral polyneuropathy, and developmental delay, with clinical and biochemical findings supporting a diagnosis of hypophosphatasia (HPP). The patient had received ongoing physical therapy since birth, and had never been able to walk without support and used a wheelchair full time. The patient was unable to eat on her own, was G-tube dependent and able to self-feed only sips of milk, and showed both receptive and expressive language delay.

The initial endocrine evaluation was part of a multidisciplinary muscular dystrophy clinic, where the patient did not say a single word during the entire visit and only used a computer-based communication device. Multiple doctors confirmed essentially no verbal output with the exception of a few single words that the patient was able to repeat during the neurological evaluation. The patient used a pulmicort nebulizer. The initial laboratory findings were: ALP 149 (normal 150-420 U/L); PLP 172.4 (normal range 20-125 nmol/L); and urinary PEA 180 (normal 0-106 nmol/mgCr).

Subcutaneous asfotase alfa injections at 6 mg/kg/week were begun three months after the patient's initial visit. At the three month follow up appointment, the parents reported that since starting the treatment, the patient appeared to have more strength when standing, and had graduated from a

wheelchair by starting to use a walker. The patient showed improvement in the ability to weight bear, although the patient still required significant support. The patient was able to move her legs and showed some use of upper extremities with fairly good strength. Overall, improved postural control of the patient's trunk and neck was noted. These improvements were attributed to the patient's overall improvement in overall muscle tone and in muscle strength, confirming the hypothesis generated by the *in vitro* murine data.

Multiple medical professionals and the parents noted a marked increase in the patient's speech. Improvement was also noted in the patient's overall language acquisition, including using more words, and putting words together to form simple sentences. The patient's increased speaking ability could also be a result of improved muscle tone and strength in response to treatment.

Specifically, the 3 months follow up appointment noted the following improvements after asfotase alfa treatment: improved rate of growth (5.8 cm/year, compared to 1.3 cm/year prior to treatment); improved strength; improved speech, i.e., saying words spontaneously and even forming simple sentences; and improved bone mineral density by 0.5 SD within the lumbar spine.

The patient had lost two teeth in the month prior to starting asfotase alfa treatment. In radiographic findings, X-rays of the wrists showed decreased bone mineralization, but were otherwise normal, X-rays of the knees showed decreased bone mineral density, gracile bones, and abnormal tibial epiphyses. The patient showed evidence of low bone mineral density. At baseline, DXA scan showed Z-scores of -4.6 and -3.3 for the lumbar spine and the total body less head, respectively. A repeat DXA scan performed 3 months after starting asfotase alfa showed an improved LBD Z-scores by 0.5 SD, although the Z-score was still low. The BMD Z-scores were -4.1 and -3.3 for the lumbar spine and the total body less head, respectively. The patient sustained an idiopathic fracture of a humerus about a month after starting treatment; treatment with asfotase alfa continued, and the fracture healed well. Renal ultrasound and eye exams were normal at baseline.

Example 4. Treating muscle weakness in patient 2

A second patient was identified as having hypotonia in conjunction with low ALP (correlated with high PPi), elevated PLP, and elevated urinary PEA. A 12 year old patient presenting with chromosomal duplication, developmental delay, autism spectrum disorder (Asperger's syndrome), and sensory processing difficulty was also noted to have a low ALP level at 90 U/L (normal range 141-460). A repeated level was again low at 91 (when tested 4 days later). The patient tired very easily, i.e., would have to rest during normal life activities involving minimal walking, and was unable to walk long distances. The patient also complained of vague pain in the shoulders, upper back, and other areas. The patient indicated that they sometimes woke up in pain and with sore shoulders and had constant pain in the legs.

The patient had no history of premature loss of teeth or of fractures. The initial occupational therapy assessment noted a fine motor score of 13 (1st percentile). Age equivalencies for response speed was 6.2 yr. Visual motor control showed a multiple year delay, i.e., at 7.9 yr level. For upper limb speed and dexterity, the patient was at 4.7 yr level. However, for teeth loss, the family history showed that one parent began to wear dentures when at 21 years old, and similarly, the maternal grandparent also had premature loss of permanent teeth and was wearing dentures at a young age. Mother's ALP

level was found to be 50 U/L (reference range for the lab 40-150). The patient's initial laboratory findings were: ALP 82 (normal 150-420 U/L); PLP 210 (normal range 20-125 nmol/L); and urinary PEA 46 (normal 0-44 nmol/mgCr).

While the patient had no history of premature tooth loss, several family members did show premature tooth loss. Radiographic analysis showed normal wrist and knee x-rays. Bone mineral density analysis via DXA scan showed normal BMD (L1-L4 Z-score 2.1, TBLH Z-score 2.4). The patient had no fracture history. The patient reported pain present in multiple sites at variable times, including frequent leg pain sufficient to disturb sleep, pain on plantar surface of feet with 5-10 minutes of standing/walking, tightness/pain in quads with fatigue, bilateral knee pain, and vague shoulder/thoracic pain. Renal ultrasound did not reveal nephrocalcinosis. The patient consistently measured at the 90% percentile for height and at the 92% percentile for weight.

The patient had no significant physical activity impairments when fully rested, however, demonstrated quad fatigue with impaired knee control and bilateral foot slap due to dorsiflexion weakness after ambulating more than two minutes. The patient was able to walk on toes, but demonstrated significant compensations when attempting to ambulate on heels. The patient's initial six minute walk test was 320 meters, significantly below the age/gender norm = 672 + 55 meters. Patient required two standing rest breaks leaning on wall secondary to fatigue. Patient demonstrated gradually increased gait impairments, including quad fatigue and foot slap due to dorsiflexion weakness, and required seated rest break following the 6MWT. The patient had trouble with exercise and became winded easily.

The patient began subcutaneous asfotase alfa injections at 6 mg/kg/week and was re-evaluated after 4 months of treatment. Overall, the patient showed multiple improvements, including in strength, agility, and endurance. The patient's 6 minute walk test improved to 597 meters (from the initial value of 320 meters). Overall, after treatment, the patient had much less pain (score of 2 out of 10, instead of 5 out of 10 reported during the previous visit) and improved mobility. These improvements were attributed to the patient's overall improvement in overall muscle tone and in muscle strength, confirming the hypothesis generated by the *in vitro* murine data.

OTHER EMBODIMENTS

All publications, patents, and patent applications mentioned in the above specification are hereby incorporated by reference to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety. Various modifications and variations of the described methods, pharmaceutical compositions, and kits of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the claimed invention. Although the disclosure has been described in connection with specific embodiments, it will be understood that it is capable of further modifications and that the invention as claimed should not be unduly limited to such specific embodiments.

CLAIMS

1. A method of treating or ameliorating a muscle weakness in a human subject having or being prone to a muscle weakness disease, comprising administering to said subject a therapeutically effective amount of at least one recombinant polypeptide having alkaline phosphatase activity.

2. The method of claim 1, wherein said subject has an elevated serum concentration of pyrophosphate (PPi) and/or low alkaline phosphatase activity.

3. The method of any one of the preceding claims, wherein a muscle of said subject is not significantly different from a muscle of a normal subject without said muscle weakness disease in at least one property selected from muscle fiber type proportion and fiber contractile properties.

4. The method of any one of the preceding claims, wherein the muscle is at least one type of leg muscles, particularly at least one type of muscles selected from soleus and extensor digitorum longus (EDL) muscles.

5. The method of claim 1, wherein said muscle weakness disease is caused by an elevated concentration of pyrophosphate (PPi) and/or low alkaline phosphatase activity.

6. The method of claim 1, wherein an elevated concentration of pyrophosphate (PPi) enhances muscle weakness in said subject.

7. The method of claim 1, wherein the muscle weakness disease is at least one of hypophosphatasia (HPP), calcium pyrophosphate dihydrate crystal deposition (CPPD), and familial hypophosphatemia.

8. The method of claim 7, wherein the familial hypophosphatemia comprises at least one of autosomal dominant hypophosphatemic rickets (ADHR), autosomal recessive hypophosphatemic rickets, X-linked hypophosphatemic rickets, and X-linked hypophosphatemia (XLH).

9. The method of claim 1, wherein the at least one recombinant polypeptide having alkaline phosphatase activity reduces the concentration of pyrophosphate (PPi) in said subject.

10. A method, comprising:

(i) identifying a population of subjects having or being prone to a muscle weakness disease;

(ii) identifying a subpopulation of subjects among the population in step (i) wherein:

(a) the subjects in said subpopulation have an elevated serum concentration of pyrophosphate (PPi) and/or low alkaline phosphatase activity;

(b) an elevated concentration of pyrophosphate (PPi) enhances muscle weakness in the subjects in said subpopulation; or

(c) (a) and (b); and

(iii) treating or ameliorating at least one symptom of a subject in the subpopulation in step (ii), comprising administering to said subject a therapeutically effective amount of at least one recombinant polypeptide having alkaline phosphatase activity.

11. The method of claim 10, wherein the muscle of the subject in step (iii) is not significantly different from the muscle of a normal subject without said type of muscle weakness in at least one property of muscle fiber type proportion and fiber contractile properties.

12. The method of claim 11, wherein the muscle is at least one type selected from soleus and extensor digitorum longus (EDL) muscles.

13. The method of claim 10, wherein said type of muscle weakness is caused by an elevated concentration of inorganic pyrophosphate (PPi) and/or low alkaline phosphatase activity.

14. The method of claim 10, wherein an elevated concentration of pyrophosphate (PPi) enhances muscle weakness in said subject in step (iii).

15. The method of claim 10, wherein said subject in step (iii) has or is prone to at least one of hypophosphatasia (HPP), calcium pyrophosphate dihydrate crystal deposition (CPPD), and familial hypophosphatemia.

16. The method of claim 15, wherein the familial hypophosphatemia comprises at least one of autosomal dominant hypophosphatemic rickets (ADHR), autosomal recessive hypophosphatemic rickets, and X-linked hypophosphatemic rickets, and X-linked hypophosphatemia (XLH).

17. The method of claim 10, wherein the at least one recombinant polypeptide having alkaline phosphatase activity reduces the concentration of pyrophosphate (PPi) in said subject.

18. The method of any one of previous claims, wherein the at least one recombinant polypeptide having alkaline phosphatase activity is administered to the subject daily for at least one week, one month, three months, six months, or one year.

19. The method of any one of previous claims, wherein the at least one recombinant polypeptide having alkaline phosphatase activity is administered by at least one of subcutaneous, intravenous, intramuscular, sublingual, intrathecal, and intradermal routes.

20. The method of claim 19, wherein the at least one recombinant polypeptide having alkaline phosphatase activity is administered subcutaneously.

21. The method of any one of previous claims, wherein the at least one recombinant polypeptide having alkaline phosphatase activity comprises at least one of a tissue nonspecific alkaline phosphatase (TNALP), a placental alkaline phosphatase (PALP), a germ cell alkaline phosphatase (GCALP), an intestinal alkaline phosphatase (IALP), and biologically functional fragments, fusions, or chimeric constructs thereof.

22. The method of claim 21, wherein the at least one recombinant polypeptide having alkaline phosphatase activity comprises at least one of a soluble fragment of TNALP, PALP, GCALP, and IALP.

23. The method of claim 21, wherein the tissue nonspecific alkaline phosphatase (TNALP) comprises an amino acid sequence of the amino acids 1-485 of SEQ ID NO: 1.

24. The method of any one of previous claims, wherein the at least one recombinant polypeptide having alkaline phosphatase activity is a fusion protein.

25. The method of any one of previous claims, wherein the at least one recombinant polypeptide having alkaline phosphatase activity comprise an immunoglobulin molecule.

26. The method of claim 25, wherein the immunoglobulin molecule is a fragment crystallizable region (Fc).

27. The method of claim 26, wherein the Fc comprises an amino acid sequence of SEQ ID NO: 20.

28. The method of any one of claims 1-27, wherein the at least one recombinant polypeptide having alkaline phosphatase activity comprises a negatively charged peptide.

29. The method of claim 28, wherein the negatively charged peptide comprises at least one of D_{10}, D_{16}, E_{10}, and E_{16}.

30. The method of claim 1 or 10, wherein the at least one recombinant polypeptide having alkaline phosphatase activity comprises a bone targeted alkaline phosphatase comprising a polypeptide having the structure:

Z-sALP-Y-spacer-X-W_n-V,

wherein sALP is the extracellular domain of the alkaline phosphatase;

V is absent or is an amino acid sequence of at least one amino acid;

X is absent or is an amino acid sequence of at least one amino acid;

Y is absent or is an amino acid sequence of at least one amino acid;

Z is absent or is an amino acid sequence of at least one amino acid; and

W_n is a polyaspartate or a polyglutamate wherein n=10 to 16.

31. The method of claim 30, wherein the spacer comprises a fragment crystallizable region (Fc).

32. The method of claim 31, wherein the Fc comprises an amino acid sequence of SEQ ID NO: 20.

33. The method of claim 30, wherein the at least one recombinant polypeptide having alkaline phosphatase activity comprises a structure of sALP-Fc-D_{10}.

34. The method of claim 30, wherein the at least one recombinant polypeptide having alkaline phosphatase activity comprises a dimer comprising monomers of an amino acid sequence of SEQ ID NO: 1.

35. The method of claim 34, wherein the at least one recombinant polypeptide having alkaline phosphatase activity is administered in a dosage from about 0.1 mg/kg/day to about 20 mg/kg/day, or a comparable weekly dosage.

36. The method of claim 34, wherein the at least one recombinant polypeptide having alkaline phosphatase activity is administered in a dosage from about 0.5 mg/kg/day to about 20 mg/kg/day, or a comparable weekly dosage.

37. The method of claim 34, wherein the at least one recombinant polypeptide having alkaline phosphatase activity is administered in a dosage from about 0.5 mg/kg/day to about 10 mg/kg/day, or a comparable weekly dosage.

38. The method of claim 34, wherein the at least one recombinant polypeptide having alkaline phosphatase activity is administered in a dosage from about 1 mg/kg/day to about 10 mg/kg/day, or a comparable weekly dosage.

39. The method of any one of the previous claims, wherein the at least one recombinant polypeptide having alkaline phosphatase activity is asfotase alfa and is administered subcutaneously at a dosage of 6 mg/kg/week.

40. The method of any one of claims 1 to 39, wherein, prior to administration of the at least one recombinant polypeptide having alkaline phosphatase activity, the subject is characterized as having an average walking distance in six minutes of about 350 meters or less.

41. The method of any one of claims 1 to 40, wherein administration of the at least one recombinant polypeptide having alkaline phosphatase activity results in an increase in an average walking distance in six minutes of at least 100 meters or more.

42. The method of any one of claims 1 to 41, wherein the subject exhibits an average walking distance in six minutes of about 500 meters or more after administration of the at least one recombinant polypeptide having alkaline phosphatase activity.

43. The method of any one of claims 1 to 42, wherein the subject exhibits decreased reliance on an assistive mobility device after administration of the at least one recombinant polypeptide having alkaline phosphatase activity.

44. The method of claim 43, wherein the assistive mobility device is at least one device selected from the group consisting of a walker, a wheelchair, braces, crutches, and orthotics.

45. The method of any one of claims 1 to 44, wherein, prior to administration of the at least one recombinant polypeptide having alkaline phosphatase activity, the subject is characterized as having a plasma PPi concentration of about 4.5 μM or greater.

46. The method of any one of claims 1 to 45, wherein administration of the at least one recombinant polypeptide having alkaline phosphatase activity results in a median decrease in PPi concentration in a plasma sample from the patient of at least about 1 μM.

47. The method of any one of claims 1 to 46, wherein the subject exhibits a plasma PPi concentration of about 2 μM to about 5 μM after administration of the at least one recombinant polypeptide having alkaline phosphatase.

48. The method of any one of claims 1 to 47, wherein, prior to administration of the at least one recombinant polypeptide having alkaline phosphatase activity, the subject is characterized as having a plasma ALP concentration of about 90 U/L or less for a subject of 0 to 14 days of age; about 134 U/L or less for a subject of 15 days of age to less than 1 year of age; about 156 U/L or less for a subject of about 1 year of age to less than 10 years of age; about 141 U/L or less for a subject of about 10 years of age to less than about 13 years of age; about 62 U/L or less for a female subject of about 13 years of age to less than about 15 years of age; about 127 U/L or less for a male subject of about 13 years of age to less than about 15 years of age; about 54 U/L or less for a female subject of about 15 years of age to less than about 17 years of age; about 89 U/L or less for a male subject of about 15 years of age to less than about 17 years of age; about 48 U/L or less for a female subject of about 17 years of age or older; or about 59 U/L or less for a male subject of about 17 years of age or older.

49. The method of any one of claims 1 to 48, wherein administration of the at least one recombinant polypeptide having alkaline phosphatase activity results in a median increase in ALP concentration in a plasma sample from the subject of at least about 100 U/L or greater.

50. The method of any one of claims 1 to 49, wherein, after administration of the at least one recombinant polypeptide having alkaline phosphatase, the subject exhibits a plasma ALP concentration of about 273 U/L or greater for a subject of 0 to 14 days of age; about 518 U/L or greater for a subject of 15 days of age to less than 1 year of age; about 369 U/L or greater for a subject of about 1 year of age to less than 10 years of age; about 460 U/L or greater for a subject of about 10 years of age to less than about 13 years of age; about 280 U/L or greater for a female subject of about 13 years of age to less than about 15 years of age; about 517 U/L or greater for a male subject of about 13 years of age to less than about 15 years of age; about 128 U/L or greater for a female subject of about 15 years of age to less than about 17 years of age; about 365 U/L or greater for a male subject of about 15 years of age to less than about 17 years of age; about 95 U/L or greater for a female subject of about 17 years of age or older; or about 164 U/L or greater for a male subject of about 17 years of age or older.

51. The method of any one of claims 1 to 50, wherein, prior to administration of the at least one recombinant polypeptide having alkaline phosphatase activity, the subject is characterized as having an average Bruininks-Oseretsky Test of Motor Proficiency 2nd Edition (BOT-2) strength score of about 10 or less.

52. The method of any one of claims 1 to 51, wherein administration of the at least one recombinant polypeptide having alkaline phosphatase activity results in an average BOT-2 strength score of the subject of about 10 or more.

53. The method of any one of claims 1 to 52, wherein, prior to administration of the at least one recombinant polypeptide having alkaline phosphatase activity, the subject is characterized as having an average BOT-2 running speed and agility score of about 5 or less.

54. The method of any one of claims 1 to 53, wherein administration of the at least one recombinant polypeptide having alkaline phosphatase activity results in an average BOT-2 running speed and agility score of the subject of about 5 or more.

55. The method of any one of claims 1 to 54, wherein, prior to administration of the at least one recombinant polypeptide having alkaline phosphatase activity, the subject is characterized as having an average Childhood Health Assessment Questionnaire (CHAQ) index score of about 0.8 or more.

56. The method of any one of claims 1 to 55, wherein administration of the at least one recombinant polypeptide having alkaline phosphatase activity results in an average CHAQ index score of the subject of about 0.5 or less.

57. The method of any one of claims 1 to 56, wherein, prior to administration of the at least one recombinant polypeptide having alkaline phosphatase activity, the subject is characterized as having an average Pediatric Outcomes Data Collection Instrument (PODCI) score of about 40 or less.

58. The method of any one of claims 1 to 57, wherein administration of the at least one recombinant polypeptide having alkaline phosphatase activity results in an average PODCI score of the subject of about 40 or more.

59. The method of any one of claims 1 to 58, wherein, prior to administration of the at least one recombinant polypeptide having alkaline phosphatase activity, the subject is characterized as having an average Muscle Strength Grade of less than about 5.

60. The method of any one of claims 1 to 59, wherein administration of the at least one recombinant polypeptide having alkaline phosphatase activity results in an average increase in a Muscle Strength Grade of the subject of about 1 or more.

61. The method of any one of claims 1 to 60, wherein administration of the at least one recombinant polypeptide having alkaline phosphatase activity results in an average increase in a Muscle Strength Grade of the subject of about 1 or more.

62. The method of any one of claims 1 to 61, wherein, prior to administration of the at least one recombinant polypeptide having alkaline phosphatase activity, the subject is characterized as having an average Hand Held Dynamometry (HHD) value of less than about 80% of a predicted HHD value.

63. The method of any one of claims 1 to 62, wherein administration of the at least one recombinant polypeptide having alkaline phosphatase activity results in an average HHD value of the subject of about 80% or more of a predicted HHD value.

64. The method of claim 62 or 63, wherein the HHD value represents the grip strength, knee flexion, knee extension, hip flexion, hip extension, or hip abduction of the subject.

65. The method of claim 1 or 10, wherein administration of the at least one recombinant polypeptide having alkaline phosphatase activity results in an increase in an average walking distance in six minutes of at least 100 meters or more.

66. The method of claim 1 or 10, wherein the subject exhibits an average walking distance in six minutes of about 500 meters or more after administration of the at least one recombinant polypeptide having alkaline phosphatase activity.

67. The method of claim 1 or 10, wherein the subject exhibits decreased reliance on an assistive mobility device after administration of the at least one recombinant polypeptide having alkaline phosphatase activity.

68. The method of claim 67, wherein the assistive mobility device is at least one device selected from the group consisting of a walker, a wheelchair, braces, crutches, and orthotics.

69. The method of claim 1 or 10, wherein, prior to administration of the at least one recombinant polypeptide having alkaline phosphatase activity, the subject is characterized as having a plasma PPi concentration of about 4.5 μM or greater.

70. The method of claim 1 or 10, wherein administration of the at least one recombinant polypeptide having alkaline phosphatase activity results in a median decrease in PPi concentration in a plasma sample from the patient of at least about 1 μM.

71. The method of claim 1 or 10, wherein the subject exhibits a plasma PPi concentration of about 2 μM to about 5 μM after administration of the at least one recombinant polypeptide having alkaline phosphatase.

72. The method of claim 1 or 10, wherein, prior to administration of the at least one recombinant polypeptide having alkaline phosphatase activity, the subject is characterized as having a plasma ALP concentration of about 90 U/L or less for a subject of 0 to 14 days of age; about 134 U/L or less for a subject of 15 days of age to less than 1 year of age; about 156 U/L or less for a subject of about 1 year of age to less than 10 years of age; about 141 U/L or less for a subject of about 10 years of age to less than about 13 years of age; about 62 U/L or less for a female subject of about 13 years of age to less than about 15 years of age; about 127 U/L or less for a male subject of about 13 years of age to less than about 15 years of age; about 54 U/L or less for a female subject of about 15 years of age to less than about 17 years of age; about 89 U/L or less for a male subject of about 15 years of age to less than about 17 years of age; about 48 U/L or less for a female subject of about 17 years of age or older; or about 59 U/L or less for a male subject of about 17 years of age or older.

73. The method of claim 1 or 10, wherein administration of the at least one recombinant polypeptide having alkaline phosphatase activity results in a median increase in ALP concentration in a plasma sample from the subject of at least about 100 U/L or greater.

74. The method of claim 1 or 10, wherein, after administration of the at least one recombinant polypeptide having alkaline phosphatase, the subject exhibits a plasma ALP concentration of about 273 U/L or greater for a subject of 0 to 14 days of age; about 518 U/L or greater for a subject of 15 days of age to less than 1 year of age; about 369 U/L or greater for a subject of about 1 year of age to less than 10 years of age; about 460 U/L or greater for a subject of about 10 years of age to less than about 13 years of age; about 280 U/L or greater for a female subject of about 13 years of age to less than about 15 years of age; about 517 U/L or greater for a male subject of about 13 years of age to less than about 15 years of age; about 128 U/L or greater for a female subject of about 15 years of age to less than about 17 years of age; about 365 U/L or greater for a male subject of about 15 years of age to less than about 17

years of age; about 95 U/L or greater for a female subject of about 17 years of age or older; or about 164 U/L or greater for a male subject of about 17 years of age or older.

75. The method of claim 1 or 10, wherein, prior to administration of the at least one recombinant polypeptide having alkaline phosphatase activity, the subject is characterized as having an average Bruininks-Oseretsky Test of Motor Proficiency 2nd Edition (BOT-2) strength score of about 10 or less.

76. The method of claim 1 or 10, wherein administration of the at least one recombinant polypeptide having alkaline phosphatase activity results in an average BOT-2 strength score of the subject of about 10 or more.

77. The method of claim 1 or 10, wherein, prior to administration of the at least one recombinant polypeptide having alkaline phosphatase activity, the subject is characterized as having an average BOT-2 running speed and agility score of about 5 or less.

78. The method of claim 1 or 10, wherein administration of the at least one recombinant polypeptide having alkaline phosphatase activity results in an average BOT-2 running speed and agility score of the subject of about 5 or more.

79. The method of claim 1 or 10, wherein, prior to administration of the at least one recombinant polypeptide having alkaline phosphatase activity, the subject is characterized as having an average Childhood Health Assessment Questionnaire (CHAQ) index score of about 0.8 or more.

80. The method of claim 1 or 10, wherein administration of the at least one recombinant polypeptide having alkaline phosphatase activity results in an average CHAQ index score of the subject of about 0.5 or less.

81. The method of claim 1 or 10, wherein, prior to administration of the at least one recombinant polypeptide having alkaline phosphatase activity, the subject is characterized as having an average Pediatric Outcomes Data Collection Instrument (PODCI) score of about 40 or less.

82. The method of claim 1 or 10, wherein administration of the at least one recombinant polypeptide having alkaline phosphatase activity results in an average PODCI score of the subject of about 40 or more.

83. The method of claim 1 or 10, wherein, prior to administration of the at least one recombinant polypeptide having alkaline phosphatase activity, the subject is characterized as having an average Muscle Strength Grade of less than about 5.

84. The method of claim 1 or 10, wherein administration of the at least one recombinant polypeptide having alkaline phosphatase activity results in an average increase in a Muscle Strength Grade of the subject of about 1 or more.

85. The method of claim 1 or 10, wherein, prior to administration of the at least one recombinant polypeptide having alkaline phosphatase activity, the subject is characterized as having an average Hand Held Dynamometry (HHD) value of less than about 50% of a predicted HHD value.

86. The method of claim 1 or 10, wherein administration of the at least one recombinant polypeptide having alkaline phosphatase activity results in an average HHD value of the subject of about 50% or more of a predicted HHD value.

87. The method of claim 85 or 86, wherein the HHD value represents the grip strength, knee flexion, knee extension, hip flexion, hip extension, or hip abduction of the subject.

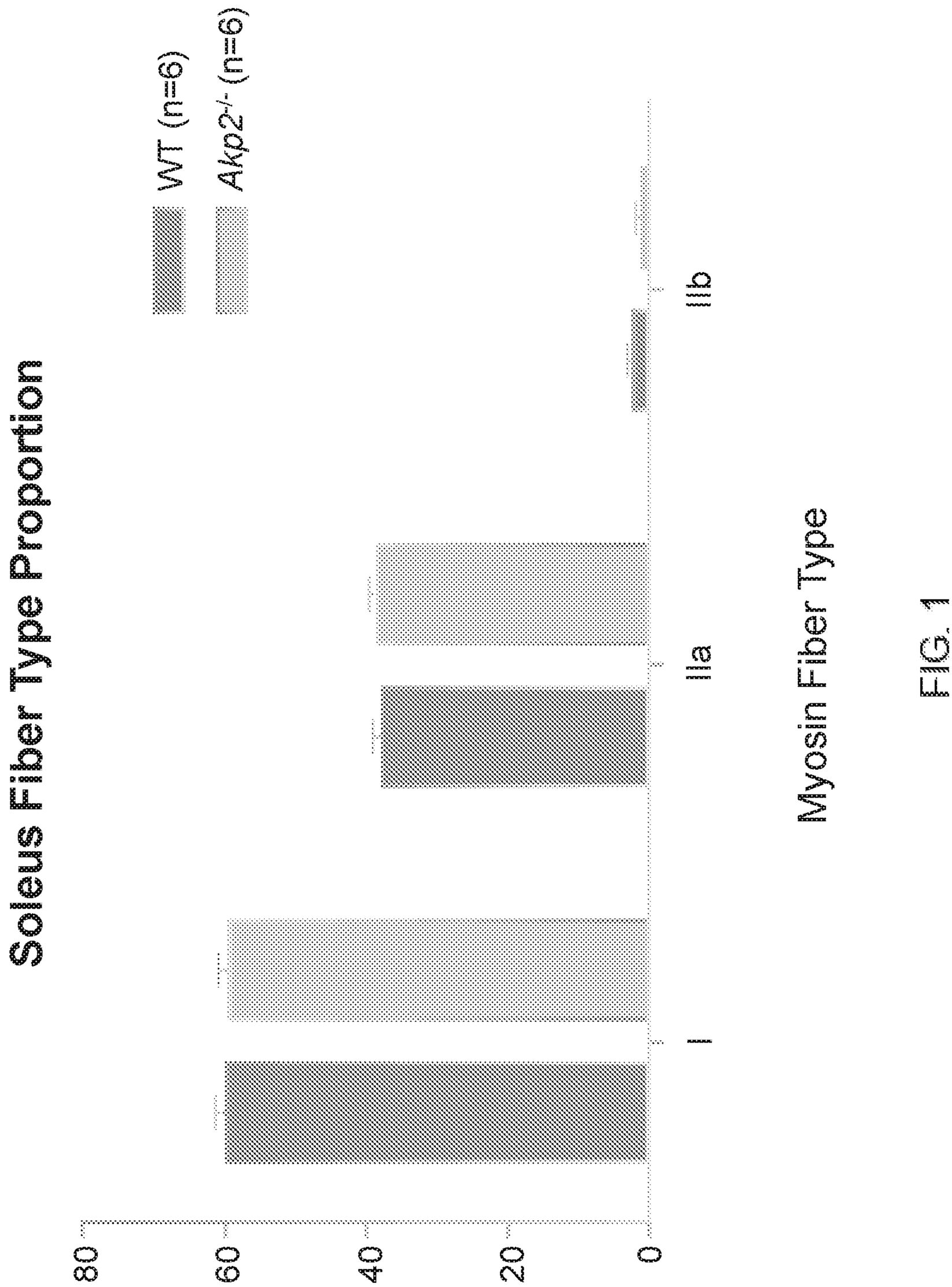

Soleus Fiber Type Proportion
WT (n=6)
Akp2-/- (n=6)
IIa
IIb
I
Myosin Fiber Type
% Fibers
80
60
40
20
0

FIG. 1

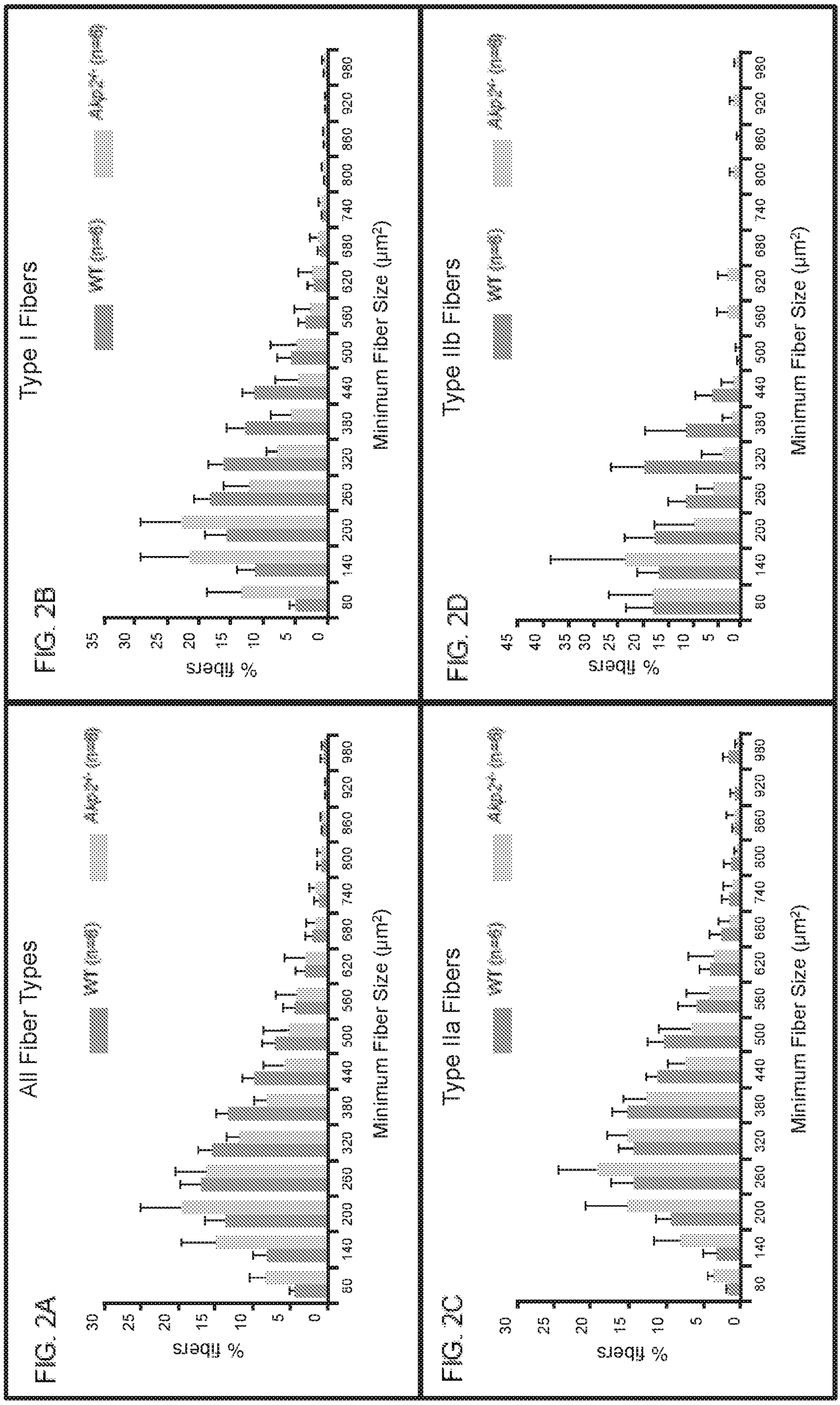
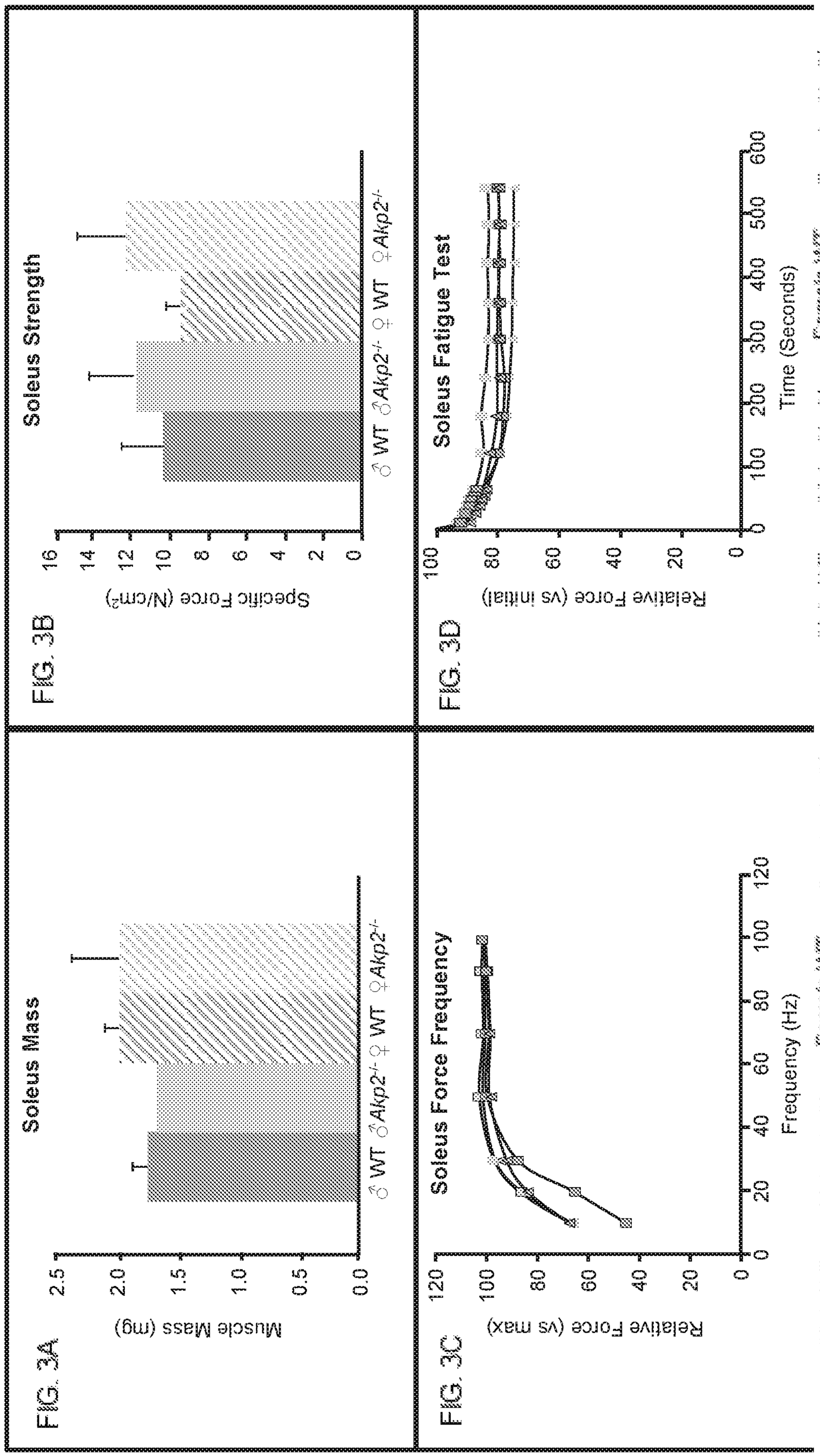


FIG. 2A
All Fiber Types
WT (n=6)
Atg7 (n=6)
% fibers
Minimum Fiber Size (μm²)
FIG. 2B
Type I Fibers
WT (n=6)
Atg7 (n=6)
% fibers
Minimum Fiber Size (μm²)
FIG. 2C
Type IIa Fibers
WT (n=6)
Atg7 (n=6)
% fibers
Minimum Fiber Size (μm²)
FIG. 2D
Type IIb Fibers
WT (n=6)
Atg7 (n=6)
% fibers
Minimum Fiber Size (μm²)


Soleus Strength
Specific Force (N/cm²)
16
14
12
10
8
6
4
2
0
♂WT ♂Akp2-/- ♀WT ♀Akp2-/-
FIG. 3B
Soleus Fatigue Test
Relative Force (vs initial)
100
80
60
40
20
0
0
100
200
300
400
500
600
Time (Seconds)
Male WT
Male Akp2-/-
Female WT
Female Akp2-/-
FIG. 3D
Soleus Mass
Muscle Mass (mg)
2.5
2.0
1.5
1.0
0.5
0.0
♂WT ♂Akp2-/- ♀WT ♀Akp2-/-
FIG. 3A
Soleus Force Frequency
Relative Force (vs max)
120
100
80
60
40
20
0
0
20
40
60
80
100
120
Frequency (Hz)
Male Akp2-/-
Female WT
Female Akp2-/-
Male WT
FIG. 3C

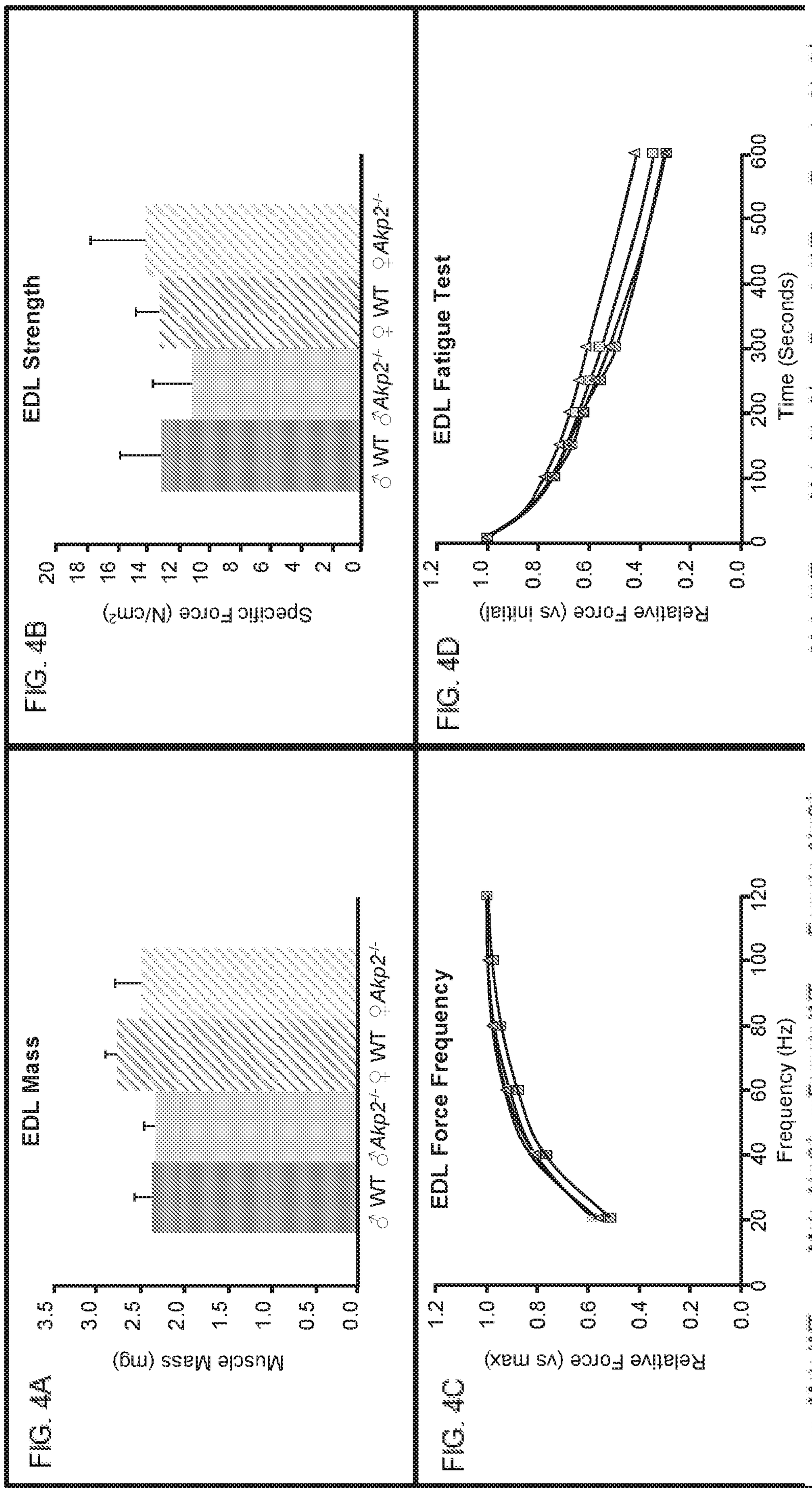

EDL Strength
Specific Force (N/cm²)
20
18
16
14
12
10
8
6
4
2
0
♂ WT ♂Akp2-/- ♀ WT ♀Akp2-/-
FIG. 4B
EDL Fatigue Test
Relative Force (vs initial)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
400
500
600
Time (Seconds)
Male WT
Male Akp2-/-
Female WT
Female Akp2-/-
FIG. 4D
EDL Mass
Muscle Mass (mg)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
♂ WT ♂Akp2-/- ♀ WT ♀Akp2-/-
FIG. 4A
EDL Force Frequency
Relative Force (vs max)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
20
40
60
80
100
120
Frequency (Hz)
Male WT
Male Akp2-/-
Female WT
Female Akp2-/-
FIG. 4C

FIG. 5A

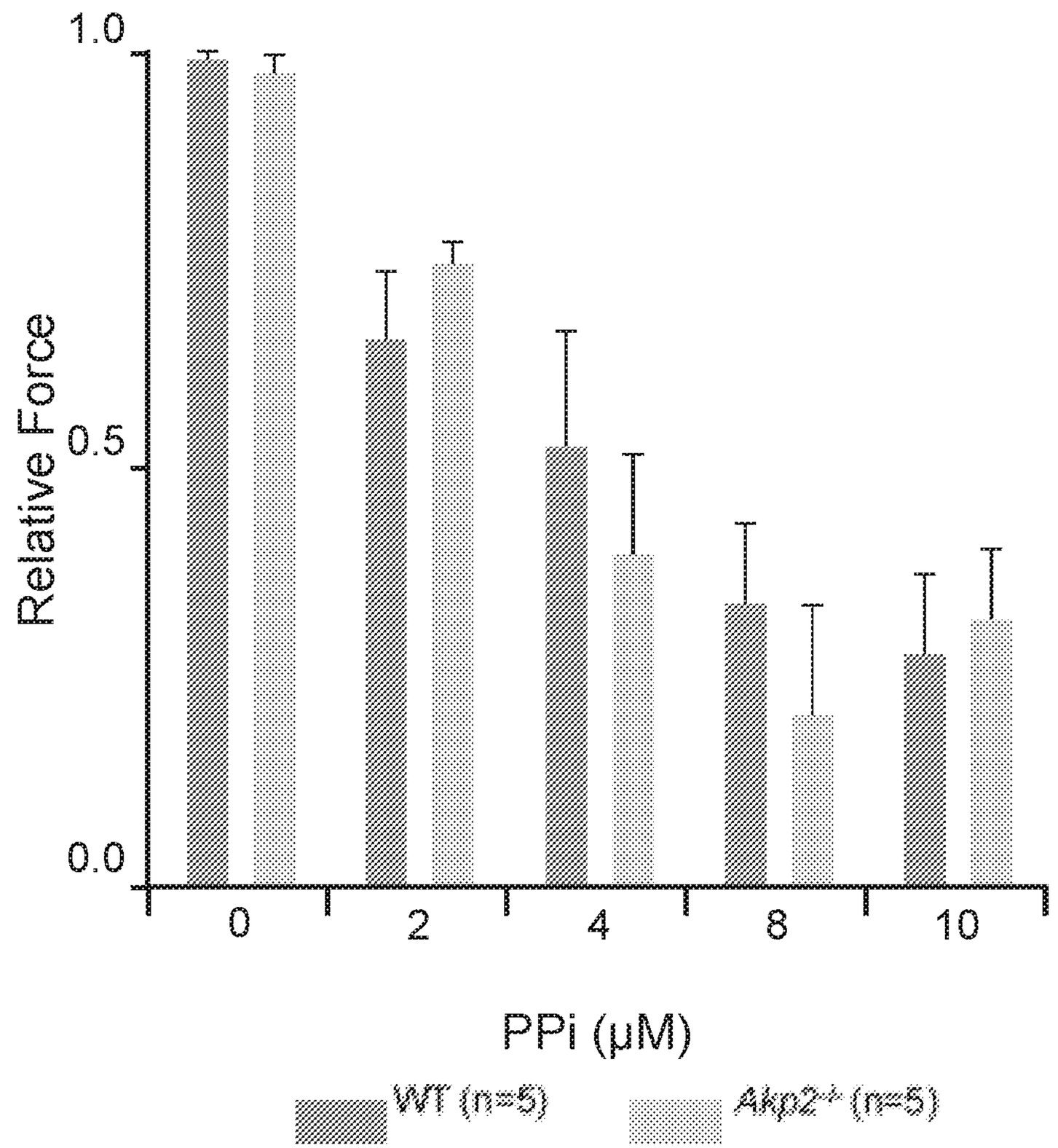
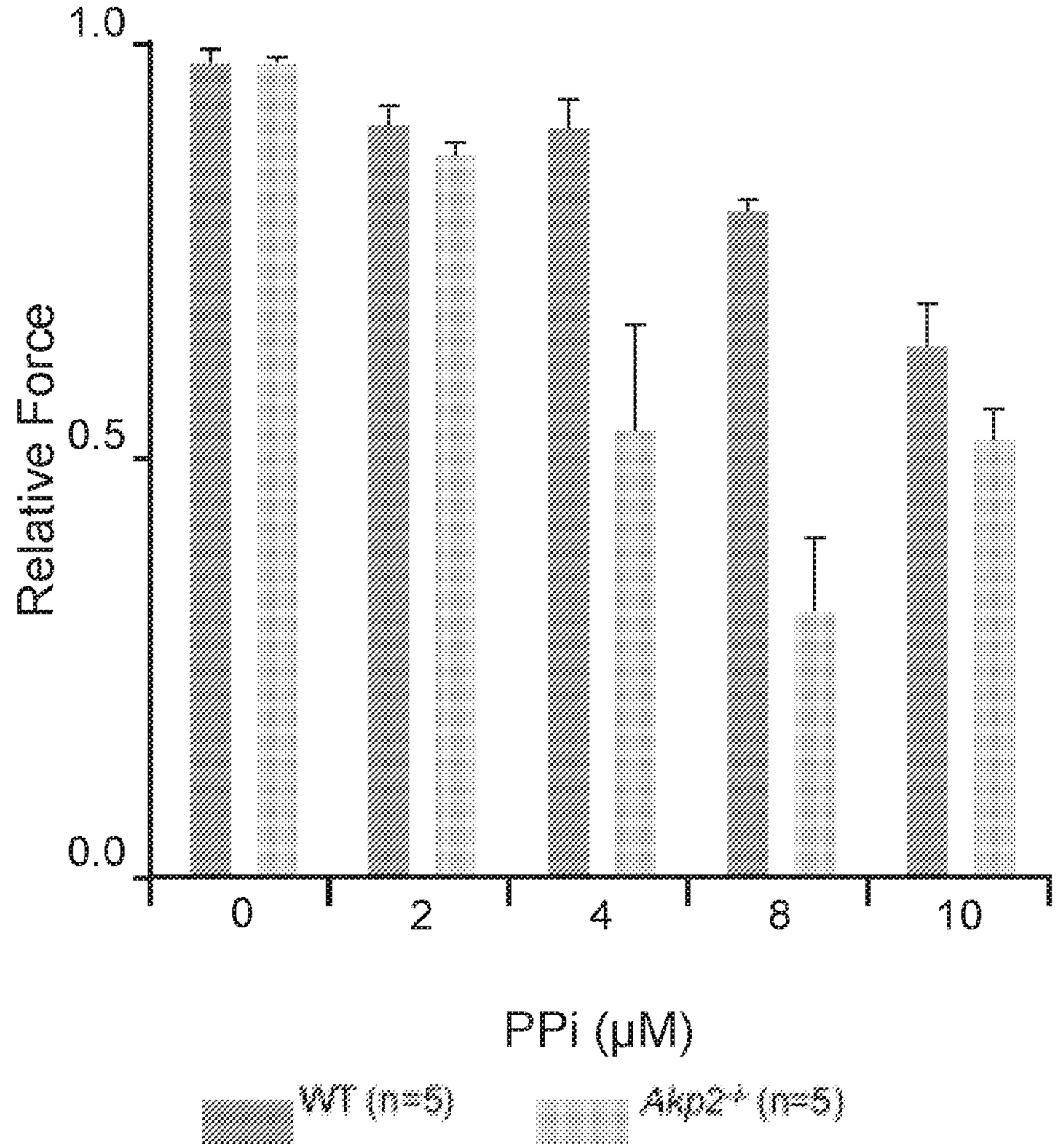

Soleus
Relative Force
1.0
0.5
0.0
0
2
4
8
10
PPi (μM)
WT (n=5)
Akp2-/- (n=5)

FIG. 5B


EDL
Relative Force
1.0
0.5
0.0
0
2
4
8
10
PPi (μM)
WT (n=5)
Akp2-/- (n=5)

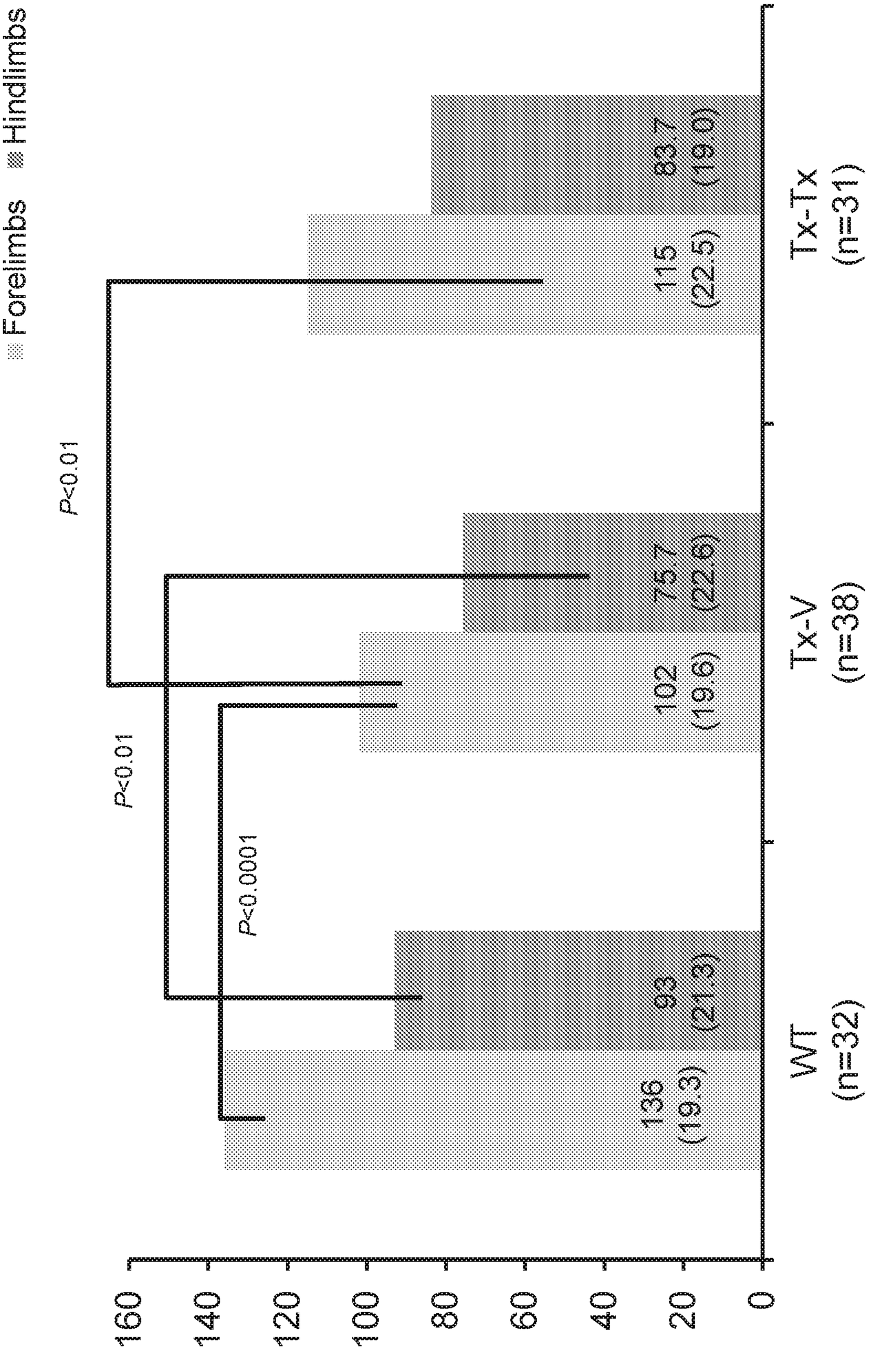

Hindlimbs
Forelimbs
P<0.01
P<0.01
P<0.0001
93.7 (19.0)
115 (22.5)
75.7 (22.6)
102 (19.6)
93 (21.3)
136 (19.3)
Tx-Tx (n=31)
Tx-V (n=38)
WT (n=32)
160
140
120
100
80
60
40
20
0
Grip strength (g)

FIG. 6

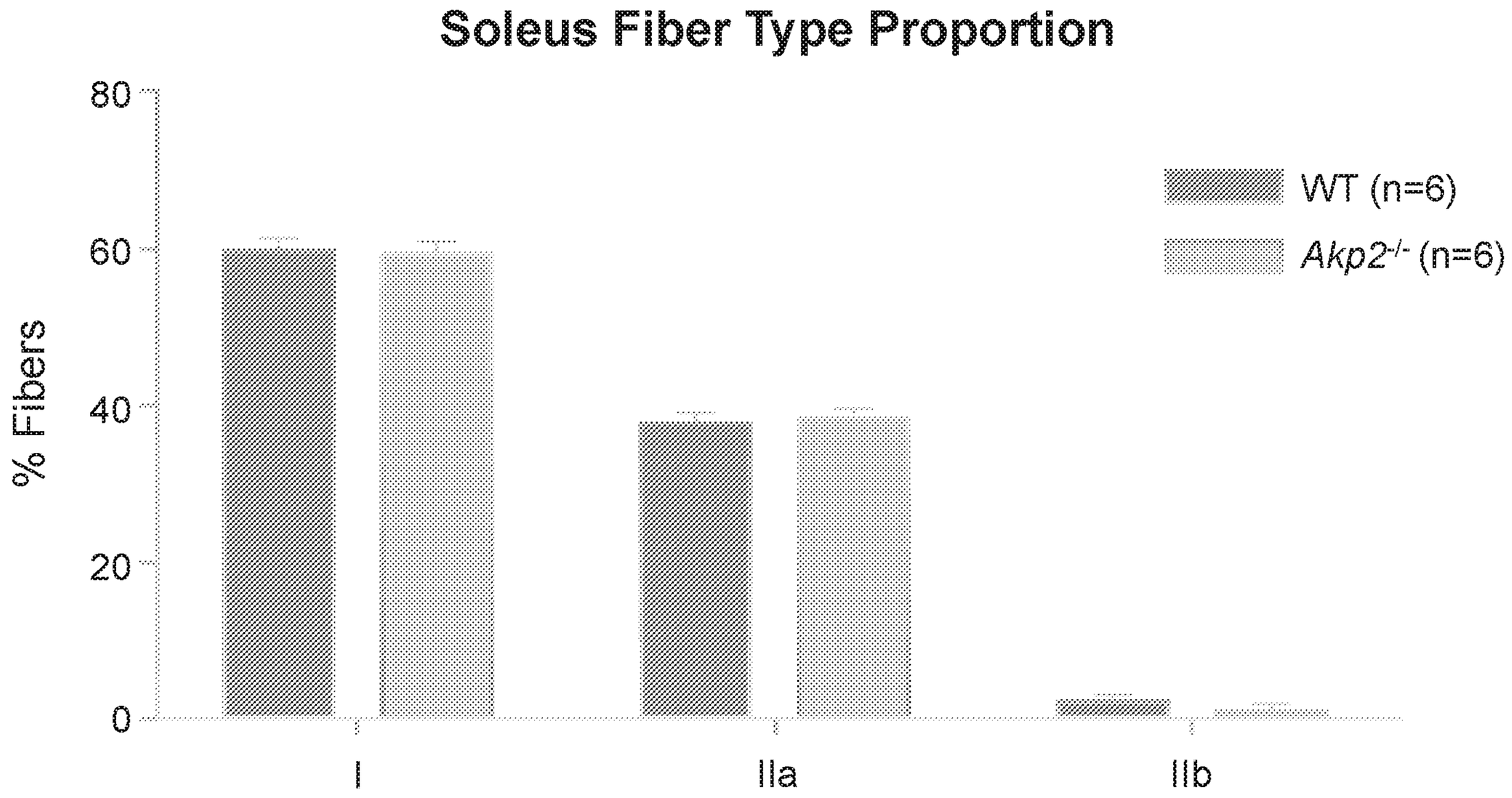

Soleus Fiber Type Proportion
80
60
40
20
0
% Fibers
WT (n=6)
Akp2-/- (n=6)
I
IIa
IIb
Myosin Fiber Type

FIG. 1