Clinical Characterization and Trial of Growth Hormone Treatment in Patients with Aggrecan Deficiency

Extension Trial Version

INVESTIGATOR-SPONSORED STUDY PROPOSAL

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3. BACKGROUND AND SIGNIFICANCE

Short stature is the most common reason for referral to a pediatric endocrinologist outside of diabetes mellitus. Current guidelines suggest performing a comprehensive evaluation of children with short stature to assess for potentially treatable conditions. As part of that evaluation, children typically undergo biochemical and hormonal testing. Most clinicians will obtain a bone age x-ray also. Despite a comprehensive evaluation, many children end up diagnosed as having idiopathic short stature (ISS). In the United States, growth hormone (GH) is approved for the indication of ISS although many insurance companies do not cover GH for ISS. The response to treatment in this group of patients is variable likely due to the heterogeneity in the true etiology of the patient's short stature.

In recent years, advances in genetic analyses have allowed us to begin to dissect the molecular etiology for short stature and re-characterize certain patients who previously would have been diagnosed with ISS. Our Growth Center has led some of these efforts, and we have established numerous novel genetic etiologies for short stature. In 2014, we described three families in which multiple members were affected by short stature with advanced bone age and premature growth cessation[1]. Using whole exome sequencing, we found that all three families had novel heterozygous mutations in the aggrecan gene (*ACAN*). Prior to this report, there had been three families in the world ever described with mutations in ACAN, each with a unique skeletal dysplasia. Our report broadened the phenotypic spectrum of ACAN deficiency and alerted the pediatric endocrinology community to this new patient population marked by short stature with advanced bone age, for whom ACAN testing is indicated.

Aggrecan, the protein encoded by the *ACAN* gene, is a critical proteoglycan component of the cartilage matrix, both in articular and growth plate cartilage, thus accounting for the dual phenotypic effects on both these types of cartilage. Consistent with this, homozygous mutations in *ACAN* in both mice and chicks severely disrupt growth plate function and impair bone elongation [2,3]. In both species, chondrocytes are tightly packed, with little intervening matrix. In chicks, there is evidence for abnormal Indian hedgehog, fibroblast growth factor, and bone morphogenetic protein signaling, leading to premature hypertrophic chondrocyte maturation[2]. Because hypertrophic differentiation induces vascular invasion and ossification of growth cartilage[4], these findings provide a likely explanation for the advanced bone age, premature growth cessation, and early epiphyseal fusion observed in individuals with *ACAN* mutations.

We published a follow up report describing 103 individuals from 20 families with confirmed mutations in *ACAN*[5]. This report describes more detailed clinical characterization of these patients. It notes some important features including a degree of subtle skeletal disproportion in many of the patients, as well as the presence of joint disease in 12 of the 20 families. We also described the growth pattern of patients with ACAN deficiency and note that there is progressive deterioration in the height standard

deviation score (SDS) because of the bone age advancement and premature epiphyseal fusion. Most relevant to the current ongoing trial, we described the response to GH treatment in 14 children treated with GH for at least 12 months. The average gain in height SDS was +0.4 after one year of treatment.

While this recent report is quite helpful to the practicing clinician, there are several key limitations. First, the patients were all assessed by different physicians at different centers. Second, the patients were treated with different GH regimens and six subjects were treated with either a gonadotropin releasing hormone (GnRH) analog or aromatase inhibitor (AI) in addition to GH. Third, all the data was collected via a retrospective chart review and thus is less reliable than prospectively collected research grade data. Finally, there was no standardized formal assessment of joint disease or the effects of GH on musculoskeletal complaints.

Given these limitations, we first proposed to perform a prospective one-year trial of GH therapy in pre-pubertal children (n=10) with documented mutations in *ACAN*. The purpose of the study was to accurately document the change in growth parameters in patients with ACAN mutations receiving GH therapy. Additionally, we performed standardized phenotyping of those patients as well as their affected family members to allow for a better understanding of the degree of joint involvement in this disorder as well as to gain insights into the underlying joint pathophysiology.

4. SPECIFIC OBJECTIVES

Primary Objective:

- Describe the effects of one year of GH therapy on growth parameters in pre-pubertal children with ACAN deficiency, followed by describing the effects of continued treatment for another two years provided the objectives for the first year of GH therapy have been reached.

Secondary Objective:

- Describe additional clinical features of ACAN deficiency, in particular the degree of joint involvement, in the affected children and their affected first-degree relatives.

5. RESEARCH DESIGN AND METHODS

Study Hypotheses:

1. Treatment with GH therapy will increase growth velocity in children with ACAN deficiency.
2. Early joint pathology will be evident on MRI examination of affected subject's knees and will provide insights into the underlying joint pathophysiology present in ACAN deficiency.

Endpoints:

A. Primary Outcome – Change in height SDS from baseline to 12 months.
B. Secondary Outcomes
    a. Height velocity in cm/year calculated over 12 months of trial.
    b. Change in height velocity. Baseline height velocity will be based on review of the medical records using the documented height closest to 12 months prior to the screening visit.
    c. Change in bone age divided by change in chronological age.
    d. Change in whole body bone mineral density adjusted for height.
    e. Frequency of adverse events as defined below
    f. Frequency of joint complaints using measures described below.
    g. Frequency of cartilage disease based on MRI evaluation of the knee as described below.

Study type

This is an open-label single arm prospective study examining the effects of a single dosing regimen of daily GH on growth response over a 12 month –followed by a four-year extension period in children with ACAN deficiency. The projected dose of GH that will be used is 50 micrograms/kg/day. This dose was initially chosen as it is within the approved range for Norditropin® for non-GH deficient states, including Turner Syndrome and Noonan Syndrome. Additionally, a parallel phenotyping study was done in affected first degree relatives of the subject.

We have elected not to have a control arm for two reasons. First, our preliminary retrospective data suggests that GH does increase HV in patients with ACAN deficiency. Additionally, the retrospective data show that patient's height SDS score will decline over time without intervention. Thus, patients and their families are highly motivated to pursue GH therapy and are unlikely to consent to be part of a study which includes a control arm. Near one-hundred percent compliance (limited missed daily GH injections) and persistence with the protocol has been observed in our cohort so far for patients at least one year on therapy and as long as 30 months. Second, this is a rare disorder, and we did not think it is feasible to recruit sufficient numbers of patients in a reasonable time frame to conduct an adequately powered randomized controlled trial.
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Study population

This study intended to recruit 10 subjects with *ACAN* mutations for inclusion in the GH treatment trial. We estimated that there could be an additional 20 affected first degree relatives who would participate in the joint phenotyping part of the study. These patients were recruited within the projected timeframe of 18 months. All subjects referred to this study will already have an identified mutation in *ACAN*; thus, we did not anticipate screen failures. This is a single-center study. All patients recruited live

throughout the United States (US). No subjects outside of the US were included in this study.

We did not require that subjects GH underwent stimulation testing to participate in this study. Subjects included in this study have a clear pathological mutation in *ACAN* explaining their short stature. Prior to the availability of this genetic test result, these individuals would have been diagnosed with ISS (or familial short stature). ACAN deficiency is a mild skeletal dysplasia similar to SHOX deficiency. There is no known (or even theoretical) link between ACAN deficiency and the possibility of GH deficiency. We do not think there is any rationale for exposing patients to the burdens of GH stimulation testing when an *ACAN* mutation has been identified as the cause of the short stature. However, to ensure that we did not include patients who may also have GH deficiency, we will only include patients with a normal IGF-I level for age and gender at the time of screening.

Inclusion Criteria

1. ACAN Deficiency – Patients must be heterozygous for a mutation in the ACAN gene. A mutation will be defined as:
    a. A heterozygous deletion of the entire gene or of ≥1 complete exons of the gene
    b. Any truncating mutation including frameshift, nonsense, splice site mutations within 2 bases of the exon/intron boundary and start loss variants.
    c. Any missense mutation which meets the following criteria:
        i. It is absent in the Exome Aggregation Consortium Database (exac.broadinstitute.org)
        ii. It is predicted to be damaging by BOTH Polyphen2 and SIFT
        iii. It segregates with the short stature phenotype in the family or is a *de novo* mutation.
    d. In-frame insertions or deletions of >1 amino acid
    e. In-frame insertions or deletions of 1 amino acid must meet the same criteria as missense mutations. For the prediction programs, Alanine will be substituted for the deleted amino acid.
    f. NOTE – Retrospective data does not show any correlation between the type of mutation and the severity of short stature. Therefore, all mutations meeting the above criteria will be included as a single group.
2. Age – Greater than or equal to 2 years 0 days. There is no upper age limit as the onset of puberty prior to treatment start will make the patient ineligible.
3. Pre-pubertal
    a. Male subjects must have a testicular volume <4 cc as determined on physical examination by a pediatric endocrinologist at the time of the screening visit.
    b. Female subjects must be Tanner 1 for breast development as determined on physical examination by a pediatric endocrinologist at the time of the screening visit.

4. Bone Age – The bone age as determined by the Greulich and Pyle method must be equal or greater than the chronological age. Bone ages will be determined at the screening visit by a single centralized radiologist.
5. IGF-I concentration level within the normal range for age and gender.
6. Ability to provide informed consent before any trial-related activities.
7. NOTE – There is no specific height standard deviation criteria for inclusion in this study. We are explicitly including patients with heights above -2.25 SDS. The reason for this is that our prior work shows that patients with ACAN deficiency have a progressive decline in height SDS due to premature growth cessation. We have documented numerous cases where individuals were initially identified with height above this threshold at a younger age and then subsequently end up severely short as they cease growing at an early age. Therefore, we do not think it is rational to apply the idiopathic short stature threshold to this specific population.

Exclusion Criteria

1. Prior treatment with any of the following therapies:
    A. Growth hormone
    B. IGF-I
    C. GnRH analog
    D. Aromatase Inhibitor
    E. Oxandrolone
2. History of any type of malignancy
3. Growth plate fusion – Defined as a bone age via the Greulich and Pyle method of 13 years in females and 15 years in males.
4. Chronic medical condition known to affect growth including but not limited to:
    A. Cystic fibrosis
    B. Diabetes
    C. Inflammatory Bowel Disease
    D. Celiac Disease
    E. Asthma requiring a daily inhaled steroid dose > 400 micrograms of inhaled budesonide per day or equivalent.
    F. Taking daily oral glucocorticoids for any reason
    G. Note – ADHD treated with a stimulant and treated hypothyroidism with a normal TSH will NOT exclude the subject from participating in the trial.
5. Malnutrition – Defined as a BMI <5th percentile (CDC growth charts)
6. Any clinically significant abnormality on screening laboratory tests as determined by the principal investigator.
7. Known or suspected allergy to trial medication, excipients, or related products.
8. Contraindications to study medications, worded specifically as stated in the product's prescribing information.
9. The receipt of any investigational drug within 90 days prior to this trial.

Withdrawal Criteria
The subject may withdraw at will at any time. The subject will be withdrawn if she becomes pregnant or intends to become pregnant. This should not be an issue as all subjects are pre-pubertal at onset of the trial.

Subject Replacement
If a subject is withdrawn from the study at any point, an additional subject will be recruited to meet our goal of 10 subjects completing 1 year of the study.

Rationale for Study Population
If a subject is withdrawn from the study at any point, an additional subject will be recruited to meet our goal of 10 subjects completing one year of the study. This has been reached. Any future drop out from the study will not be replaced by new recruitment as all 10 subjects are past the one-year treatment mark.

Subject Recruitment/Consent
Potential subjects will be identified through several different recruitment modalities. First, all eligible patients who participated in the Growth Center's prior retrospective study, 'Uncovering the Genetic Basis for Growth Disorders', will be contacted directly by Dr. Philippe Backeljauw or by Dr. Andrew Dauber. Second, the Growth Center continues to receive inquiries about the treatment of ACAN deficiency from endocrinologists and geneticists with new patients being diagnosed on a weekly basis. With each new contact, a member of the study staff will inform the physician about the trial and will provide a study brochure to be shared with the patients. Third, the study will be publicized online in a number of forums. In addition to posting the trial on clinicaltrials.gov, it will be posted on the Cincinnati Center for Growth Disorders research website, the CCHMC online trial posting, as well as on the Pediatric Endocrine Society site. Fourth, Dr. Backeljauw and Dr. Dauber will contact the most prominent genetics diagnostics labs in the United States who are performing ACAN testing and ask them to inform patients with ACAN mutations about the trial. Dr. Dauber already has an arrangement in place with GeneDx for research referrals for patients with ACAN mutations.

Once a potential subject is identified, the research team will contact the subject and provide them information about the trial. All subjects' will have their mutations confirmed in either the Cincinnati Center for Growth Disorders Lab or the Center for Genetic Medicine Research Lab at Children's National Medical Center. All first-degree relatives will be tested for the presence of the mutation. The genetic testing will be performed as part of a separate approved genetics protocol already in place at CCHMC. Potentially eligible subjects will be mailed/emailed a copy of the consent for review. The study team will be available by telephone to answer any questions about the protocol. Interested subjects will be brought to CCHMC for a screening visit. At the time of the screening visit, the consent will be re-reviewed with the subject and the appropriate family members. Written informed consent will be obtained from all eligible subjects

and appropriate family members (see below, section on Phenotyping protocol for family member eligibility). Written assent will be obtained for all subjects aged 11 years or older. Written consent will be obtained from a parent for children under age 11. Children under age 11 will not be required to provide written assent as per CCHMC IRB regulations.

Additional informed consent and assent will be obtained from all subjects meeting the criteria for continuation of GH therapy for an additional two years who agree to participate in the 'extension trial'.

Growth Hormone Trial Procedures (Year 1 Table 1)

A. Screening visit – The subject will undergo a routine physical examination including a focused joint exam (inspection of the hip joints, checking for scoliosis) and pubertal staging performed by a pediatric endocrinologist. Vital signs will be obtained. Weight will be measured on a digital scale. Height will be measured in triplicate on a standing stadiometer which is calibrated daily. The average of the three measurements will be used. A medical history will be obtained, and a detailed family history will be obtained with specific attention to growth and joint issues. A pedigree will be drawn. A funduscopic exam will be performed. Photographs will be taken of the subject. Screening labs will be performed as detailed in **Table 1**. A bone age x-ray and DXA scan (whole body and lumbar spine) will be obtained in addition to the joint phenotyping visit procedures. The subject and family members will receive education about the administration of GH from a qualified nurse. The GH dose of 50 micrograms/kg/day will be calculated based on the weight. Medication will not be provided at the visit as screening labs will be pending. We anticipate a near 100% screen positive rate given that subjects will have been pre-screened for eligibility and it will be very unusual for the subjects to have a laboratory abnormality which would disqualify them. All screening lab tests will be performed at the CCHMC clinical laboratories.
B. GH initiation – If the subject passes screening, a supply of GH therapy will be shipped to the subject from the study coordinator. The supply amount will be determined by what is needed for the 3 months +/- 2-week window until the next check-in/visit to ensure that subjects do not run out of medication. Receipt of GH will be confirmed via telephone call and the date of GH initiation will be recorded. A log will be provided to the subject to record daily compliance for the first year of treatment with GH.
C. 1-week telephone call – A telephone call will be made at 1 week (+/- 2 days) to review GH administration and address any issues with medication administration.
D. 3-month telephone call and remote lab draw – A telephone call will be made at 3 months (+/- 2 weeks) to review compliance and assess for any adverse events that may not have been previously reported (See Adverse Event reporting section). After successful completion of this call, an additional 3 month +/- 2-

week supply of GH will be shipped to the subject. A lab slip will be provided for measurement of IGF-1 level at a local lab. This value is only being used as a safety measure and not as an efficacy outcome measure and thus will not be centralized. It will logistically not be possible to centralize this sample.

E. 6-month visit – The subject will return to CCHMC 6 months (+/- 2 weeks) after initiation of GH therapy for a repeat visit. The subject will undergo a routine physical examination including a focused joint exam (inspection of the hip joints, checking for scoliosis) and pubertal staging performed by a pediatric endocrinologist. Vital signs will be obtained. Weight will be measured on a digital scale. Height will be measured in triplicate on a standing stadiometer which is calibrated daily. The average of the three measurements will be used. A funduscopic exam will be performed. Labs will be performed as detailed in **Table 1**. The compliance log will be reviewed, and the subject will be assessed for any adverse events that may not have been previously reported. The dose of GH will be weight adjusted to maintain a dose of 50 micrograms/kg/day. An additional 3 month +/- 2-week supply of GH will be provided to the subject.

F. 9-month telephone call – A telephone call will be made at 9 months (+/- 2 weeks) to review compliance and assess for any adverse events that may not have been previously reported (See Adverse Event reporting section). After successful completion of this call, an additional 3 month +/- 2-week supply of GH will be shipped to the subject.

12-month visit - The subject will return to CCHMC 12 months (+/- 2 weeks) after initiation of GH therapy for a repeat visit. The subject will undergo a routine physical examination including a focused joint exam (inspection of the hip joints, checking for scoliosis) and pubertal staging performed by a pediatric endocrinologist. Vital signs will be obtained. Weight will be measured on a digital scale. Height will be measured in triplicate on a standing stadiometer which is calibrated daily. The average of the three measurements will be used. A funduscopic exam will be performed. Labs will be performed as detailed in **Table 1**.

Table 1: Lab and Assessment Schedule for treatment year 1

Lab/Assessment	Screening Visit	3-month TC/remote lab visit	6-month visit	9-month TC	12-month visit
Physical Exam	X		X		X
Vital Signs	X		X		X
Weight	X		X		X
Height	X		X		X
CBC	X				
Renal Panel	X				
Hepatic Function Panel	X				
TSH	X		X		X
Free T4	X		X		X

IGF-1	X	X	X		X
IGFBP-3	X		X		X
Photographs	X				
Bone Age X-ray	X				X
Musculoskeletal Exam	X				
DXA scan (whole body and lumbar spine)	X				X
IKDC Knee Score	X				
QOL Survey	X				
Physical Activity Assessment (Marx)	X				
Oswestry back survey	X				
Compliance Review		X	X	X	X
Adverse Event Review		X	X	X	X
MRI	X				
Knee X-rays	X				

After each in-person study visit, we will send a letter summarizing the exam findings and lab results to the participant's local primary care physician and/or pediatric endocrinologist.

Stopping Rules and Dose Reduction Criteria

The treatment portion of the trial will be discontinued for an individual subject if he or she develops a serious adverse drug reaction (as defined below). The treatment trial may otherwise be discontinued if the subject withdraws consent or if the PI determines that the subject is no longer eligible to continue treatment.

The dose of GH will be adjusted based on weight at study visits. It will not be increased or decreased based on efficacy (or lack thereof) with the following exceptions: at the 3, 6, 18 month and any added remote lab draw, if the IGF-I exceeds +2.5 SD, the GH dose will be reduced. Reductions will be 10%- 20% at the discretion of the PI, depending on the extent of IGF-I elevation. In the unlikely event that the IGF-I remains above the +2.5 SD mark at the next check, the dose will be further decreased by either 10%-20% and an additional IGF-I level will be checked at the PI's discretion.

Joint Phenotyping Procedures for Subjects and Relatives

The goals of the joint phenotyping protocol are twofold. First, we would like to comprehensively assess the prevalence of joint complaints in this patient population with specific focus on the knee joint. Second, we would like to examine the early radiological manifestations of joint disease in these patients by using a cartilage-focused MRI without contrast protocol at the knee. These imaging studies will provide insights into the pathological effects of mutations in *ACAN* on joint cartilage. Once arthritis is

advanced, the MRI findings will no longer be specific or instructive, so we will only perform MRI assessments in individuals under age 20 years.

We are focusing our investigations on the knee joint for two reasons. First, retrospective data show that the knee joint is the most commonly involved joint in ACAN deficiency associated arthritis[5]. Second, the other joint(s) with significant pathology in ACAN deficiency are the intervertebral joints in the back. These joints have many normal variants on MRI imaging, and we think it will be difficult to determine which MRI findings represent true pathology versus normal variation. We will perform a thorough musculoskeletal evaluation through physical examination (for those who are able to do the assessment based on age and/or ability) and questionnaires which will include all joints (see below). We believe that MRI examination of the knee is most likely to provide insights into the pathophysiology of ACAN associated joint disease.

All first-degree relatives will be screened for the ACAN mutation identified in the primary subject in the family. More distant relatives will be offered inclusion in the study at the discretion of the subjects' parents and Dr. Backeljauw. This genetic testing will be performed as part of a separate genetics protocol. Any family member identified to carry the same mutation as the primary subject will be eligible for inclusion in the phenotyping protocol. If a family member meets inclusion criteria for the GH treatment trial, they also will be offered the option of enrolling in the treatment trial. Eligible family members will come to CCHMC for a single research visit where they will undergo a detailed phenotypic evaluation as detailed below. This evaluation will be scheduled to coincide with one of the primary subject's research visits. We estimate that each subject will have one affected parent and one affected individual giving us a potential total of 30 affected individuals undergoing joint phenotyping (~20 of whom will be under 20 years old).

The detailed phenotyping evaluation includes the following assessments:

1. Height – Measured in triplicate on a standing stadiometer which is calibrated daily. The average of the three measurements will be used.
2. Weight – Measured on an electronic scale.
3. Detailed musculoskeletal evaluation performed by physical therapist including the IDKC knee score (a validated knee instrument), Oswestry back survey, a 3-dimensional biomechanical and gait assessment.
4. Questionnaire regarding medical history with specific attention to growth and joint issues as well as a validated quality of life survey (Peds QL) and physical activity assessment questionnaire (Marx).
5. Photographs
6. Radiographs
    a. Standard 4 view X-ray series of the knees in all affected individuals.
    b. Subjects under 20 years of age will have a bone age performed.
7. 3T MRI of the knees will be performed on all subjects who are able to do the assessment without sedation (per Radiology generally age 6 and older) under

age 20 years to look for early evidence of joint disease. For all subjects, a standard MRI checklist will be used to assess eligibility prior to scheduling any study visits. Subjects can enroll and participate in the study even if they are not able to undergo the MRI procedure.

Specifically, we will use a combination of MRI sequences which are specifically designed to look at cartilage. We will begin with T2 relaxation time mapping (T2 mapping), a quantitative MR technique, highly sensitive to changes in collagen content and anisotropic orientation of collagen fibers within the cartilage [7,8]. T2 mapping allowed early detection of cartilage changes in JIA which was not predictable either on conventional MR or clinical exams [9,10]. Additionally, $T_1\rho$ imaging is a promising new technique, highly sensitive to the interaction of water molecules with glycosaminoglycan (GAG). By measuring collagen fiber anisotropic changes (reflected by increased T2 values) in combination with GAG depletion (reflected by increased $T_1\rho$), we can obtain a complete set of information of hyaline cartilage [11-15]. Finally, we will use diffusion tensor imaging (DTI), a noninvasive technique enables measurement of the Brownian motion of water to show tissue microstructure. DTI based tractography has enabled probing of the tissue finer orientation from voxel to voxel in three dimensions. There has been a research trial using DTI to show the arrangement and measurement of the length of the columns and microstructures of the growth plate [16].

The purpose of the MR imaging of the knee is:

1. Evaluation of early degeneration of the knee joint including gross morphologic changes (conventional MR) as well as microstructure changes of the cartilage (quantitative MR).
2. Evaluation of growth plate abnormalities by measuring track length of the columnar cells of the growth plate (DTI).

The purpose of obtaining the MRI is not to assess the effects of GH on the joint disease. Our study will be underpowered to perform this assessment and as MRI evaluations are costly, we will only perform the MRI once at the baseline visit. This will be sufficient for the scientific purposes stated above.

As the initial phenotype arm of the study reached completion in November 2019, our data showed that osteochondritis dissecans (OD) was present on knee MRI in 25% of pediatric patients. These subjects ranged from 8-12 years old. In order to gain further insight into the natural progression of OD and other joint pathology throughout childhood, we propose to repeat knee MRIs for those that had OD on their initial imaging. Repeat MRIs will be done every other year from the time of the initial study. The benefit appears to greatly outweigh any risks, as this would not expose any of the study subjects to additional radiation.

Written informed consent will be obtained from all subjects undergoing the phenotyping protocol and written assent will be obtained in all minor subjects aged 11 years or older.

6. EXTENSION TRIAL

Background and Preliminary Results

As stated in our initial study protocol, patients demonstrating a year-1 increase in height SDS > + 0.3 will qualify to enroll in the extension trial, which will allow continuation of GH therapy for another two years, while using the same GH dosing protocol as for year 1. Of the five patients receiving GH therapy for at least six months, results from their 6-month study visits yield a favorable growth response. The annualized mean height velocity for this group is 9.4 cm/yr (range, 7.4 to 11.2 cm/yr). The annualized mean increase in height SDS is + 0.8 (range, + 0.5 to + 1.4). Even the lowest responder achieved a gain in height SDS of > +0.3 (namely, + 0.5 SDS), and thus qualifying for further treatment with GH as part of the study's extension trial requirements. All families have shown good practice adherence with both the GH injections, and the follow-up study visits so far. At this point, GH therapy is not FDA-approved for ACAN deficiency associated growth failure. There are, to our knowledge, no other trials for this disorder available to the patients with ACAN deficiency.

Specific Objective for the extension trial

Primary objective:

- Describe the continued effects (beyond year 1) of GH therapy on growth parameters in pre-pubertal children with ACAN deficiency.

Secondary objective:

- Continue to describe features of joint involvement, as noted on physical exam or history, in patients with ACAN deficiency receiving continued GH treatment - to provide additional insights into the underlying joint pathophysiology present in ACAN deficiency.

Study Design and Methods

Study Hypotheses:

1. Continued treatment with GH in children with ACAN deficiency will result in ongoing catch-up growth due to increased growth velocity.

2. Early joint pathology demonstrated on prior MRI will be evident on clinical examination of affected subjects.

Endpoints:

A. Primary Outcome – Change in height SDS from 12 to 36 months, and overall delta height SDS from start of therapy to 36 months.

B. Secondary Outcomes
    a. Height velocity in cm/year calculated for each year of the trial.
    b. Change in height velocity during years 2 and 3 of GH therapy.
    c. Change in bone age divided by change in chronological age for each year of continued therapy.
    d. Change in whole body bone mineral density adjusted for height at 24 and 36 months.
    e. Frequency of adverse events as defined below.
    f. Frequency of joint complaints.

Study Recruitment

Only study participants that have had a satisfactory response to GH within the first year of treatment will have the opportunity to enroll in the extension trial. This is defined as an increase of height SDS of + 0.3 or greater.

Study protocol

This is an extension trial of rhGH therapy in pre-pubertal children with ACAN deficiency. The primary outcome measures of interest are height velocity and change in height SDS. All patients currently in the treatment trial will continue to receive once daily injections of Norditropin at a standard dose of 50 mcg/kg/day, per initial study protocol (this dose can only be adjusted based on safety guidelines as described in the original protocol for year 1 of therapy, i.e., if IGF-I concentration on therapy is > +2.5 SD)

Follow up visits will occur every 6 months, or semi-annually. Study staff will continue to perform check-in calls every 3 months to promote compliance and to record any missed doses, potential new adverse events or use of concomitant medications. In addition, study staff will dispense an additional 3 months of medication and supplies after the 3-month check confirms adequate adherence with the study protocol and no new treatment-related adverse events were reported. Each follow-up visit has a +/-2-week window to accommodate participant schedules and travel. These visits will be the same as previous ones performed for the initial 12-month trial.

The chart below outlines the visit activity schedule **for the extension trial**.

Lab/Assessment	**Check-in Calls**	**18- and 30-Month Visits**	**24- and 36-Month Visits**
Physical exam		X	X
Vital signs		X	X
Height/weight		X	X
TSH		X	X

Free T4		X	X
IGF-I		X	X
IGFBP-3		X	X
Bone Age X-ray			X
DXA scan (whole body, lumbar spine) *			X
Compliance Review	X		
Adverse Event Review	X		
Medication Dispensation	X	X	X

*Additional radiation will be received, approx. 0.013mSv per scan

Only if a participant was unable to complete a study procedure during the initial 12-month trial period due to age, scheduling conflicts, or illness, they may be asked to perform the procedure during the extension trial at one of their scheduled study visits (i.e., MRI, gait assessment, musculoskeletal evaluation).

After each study visit, we will send a letter summarizing the exam findings and lab results to the participant's local primary care physician or pediatric endocrinologist.

Reasons for Completion of the Clinical Trial Participation

When it becomes clear that a participant is no longer benefitting from ongoing GH therapy, treatment will stop.

To discontinue further GH therapy during the extension trial, either of the following must occur:

1. The participant reaches skeletal maturity according to their most recent bone age x-ray.
2. The participant has a height velocity of <2.5 cm/yr.
3. The participant and/or his/her family feels continued therapy does not lead to satisfactory treatment response.
4. The participant has completed their last study visit (3 year) and there is no more funding to continue the trial.

The treatment portion of the trial will be discontinued for an individual subject if he or she develops a serious adverse drug reaction (as defined below). The treatment trial may otherwise be discontinued if the subject withdraws consent or if the PI determines that the subject is no longer eligible to continue treatment. Participants that discontinue the treatment portion of the trial will be encouraged to follow up with their local pediatric endocrinologist for follow-up care and management.

If a subject's participation (and treatment) ends due to 1) completion of the trial and/or 2) lack of continued funding, study staff will work with the family to establish clinical care and GH treatment through a local pediatric endocrinologist. Up to 3 months' worth of medication and supplies can be dispensed to the family at the last study visit to ensure continuity of ongoing care during this transition period.

7. REGULATORY CONSIDERATIONS

In consultation with Novo Nordisk, we have determined that the study will continue to be conducted under an IND. Dr. Backeljauw, as the current sponsor, will continue to have the IND with the FDA and will comply with all necessary regulations. In the event Dr. Backeljauw should leave his position at CCHMC, the newly appointed PI will take over the IND and assume all responsibilities that go with it.

8. RISKS/BENEFITS

The primary benefit of this study is the expected increase in growth rate in individuals treated with GH therapy. Subjects will be provided with a copy of their growth data and growth chart at each visit. Additionally, after each visit, a letter will be sent to the subject's primary care physician detailing the exam findings and laboratory results from that visit. Additionally, this study will provide us additional insights into the clinical presentation of ACAN deficiency which may benefit subjects and their family members in the future.

The major risks from this trial are related to the treatment with GH itself. Known side effects of this FDA-approved medication include mild edema, intracranial hypertension, slipped capital femoral epiphysis (SCFE), progression of scoliosis, and injection site reactions.

- Edema: Edema is usually mild and self resolves. Subjects will be asked about foot swelling. If edema persists and is painful, the dose of GH will be decreased 10-20%. If the edema continues to persist 2 weeks after decreasing the dose and is impacting the subject's daily activities, GH will then be discontinued.
- Intracranial hypertension: A funduscopic exam will be performed at baseline and at each follow up visit. In the case of persistent headache +/- blurred vision or vomiting, the patient will be referred to a local ophthalmologist for dilated eye exam to rule out papilledema. If papilledema is present, the subject will then be referred to a local emergency department for further evaluation including lumbar puncture. If papilledema is present, GH will be immediately discontinued. After the complete resolution of all headache symptoms and the complete resolution of papilledema, GH can be restarted at a reduced dose of 30 micrograms/kg/day. If the patient tolerates this dose for 2 months without recurrence of symptoms, then the dose can be increased back to 50 micrograms/kg/day. If at any point papilledema recurs, GH will be permanently discontinued.
- SCFE: A hip exam will be performed at baseline and at each follow up visit. If the patient develops hip or knee pain at any time during the study a hip X-ray will be obtained to rule out SCFE. If SCFE is present, GH will be immediately discontinued.
- Progression of scoliosis: A back exam will be performed at baseline and at each follow up visit. If the patient develops clinically significant scoliosis during the study, the patient will be referred to an orthopedic physician for

further evaluation. The decision whether to discontinue GH will be made in conjunction with the orthopedist depending on the severity of scoliosis. Scoliosis has been reported in a single family with homozygous mutations in the ACAN gene[18]. These reported patients had a very severe form of the disease. The subjects in the current clinical trial will all have heterozygous mutations in ACAN. In our report of 103 individuals with heterozygous mutations, scoliosis was not reported in any individual. Additionally, scoliosis usually becomes more evident during puberty and all individuals in this study will be pre-pubertal. Thus, it is highly unlikely that scoliosis will develop during the trial. If it does, we will be unable to determine if the scoliosis is due to the underlying ACAN deficiency or GH treatment.

- Injection Site Reactions: The patients can experience local symptoms related to the subcutaneous administration of GH which include pain at the site of the injection, redness, small bruising, lipoatrophy or lipohypertrophy. Injection site reactions can be avoided by changing the injection site at each injection (injection site rotation).

Radiation risk: Every person is exposed on a daily basis to a certain amount of background radiation originating from soil, rocks, outer space and within the body itself. The amount of radiation for imaging studies varies based on the size of the patient, with children and smaller adolescents being exposed to relatively larger doses of radiation than adults. The average person in Cincinnati receives a radiation dose of about 3 mSv per year. Using the largest exposure estimates for children, the proposed radiology studies would expose the patients to the following amounts of radiation:

- DXA scan (whole body, lumbar spine): approx. 0.013 mSv; four DXA scans: approx. 0.052 mSv
- Bone age (X –ray of the hand): 0.005 mSv; four bone age studies: 0.02 mSv
- Knee X-rays (4 views): 0.001 mSv per view; total 0.004 mSv (for screening visit only, until all 10 patients have been enrolled; afterwards no knee-x-rays are planned for the extension part)

Therefore, total radiation exposure from this study is expected to be 0.08 mSv or less (equivalent to the amount an individual receives over 6 days from background radiation).

9. ADVERSE EVENTS

Subjects will be encouraged to notify the study team immediately should any adverse event occur. Formal review of adverse events will occur at each visit and telephone call as detailed in Table 1. All adverse events will be reported to the IRB, Novo Nordisk and to the FDA as per regulations. An adverse event log will be maintained.

Given that this continues to be a small pilot trial with an anticipated low rate of adverse events, there will be no formal data safety and monitoring board. Rather, Dr. Backeljauw or a qualified co-investigator when Dr. Backeljauw is unavailable will review

all adverse events within 48 hours and serious adverse events within 24 hours. If a subject requires discontinuation of GH therapy, they will be asked to still complete all follow up assessments up to the 3-year visit.

The study team will comply with all local legal, regulatory, and IRB requirements with regards to adverse events. Dr. Backeljauw, the sponsor-investigator, will be responsible for reporting of all adverse events including serious adverse events (SAE), suspected unexpected serious adverse reactions (SUSARs), serious adverse drug reactions (SADRs) to the competent authority and independent ethics committee/institutional review boards based upon federal regulations and local/IRB policies.

Dr. Backeljauw, the sponsor-investigator, will report to Novo Nordisk all SAEs, SUSARs, and SADRs at the same time such events are reported to regulatory authorities or within 15 days from the sponsor-investigator becoming aware of such adverse events, whichever comes first.

Dr. Backeljauw or his designee will collect the following information at minimum for each of these events:

1. Study name
2. Patient identification (e.g., initials, sex, age)
3. Event (preferably a diagnosis)
4. Drug
5. Reporter identification (e.g., Name, or initials)

Also 6) Causality, and 7) Outcome might be reported.

Definitions

Adverse Event (AE):
An AE is any undesirable medical event occurring to a subject in a clinical trial, whether or not related to the trial product(s). This includes events reported from the first trial related activity after the subject has signed the informed consent and until completion of the trial. Unexpected AEs as referenced in the US Norditropin® prescribing information.

Clinical Laboratory Adverse Event:
A clinical laboratory AE is any clinical laboratory abnormality regarded as clinically significant i.e., an abnormality that suggests a disease and/or organ toxicity and is of a severity, which requires active management, (i.e., change of dose, discontinuation of trial product, more frequent follow-up, or diagnostic investigation).

Serious Adverse Event (SAE):
A serious AE is an experience that at any dose results in any of the following:
• Death

• A life-threatening* experience
• In-patient hospitalisation or prolongation of existing hospitalization
• A persistent or significant disability/incapacity
• A congenital anomaly/birth defect
• Important medical events that may not result in death, be life-threatening*, or require hospitalisation may be considered an SAE when, based upon appropriate medical judgement, they may jeopardise the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
• Suspicion of transmission of infectious agents

*The term life-threatening in the definition of SAE refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it was more severe.

Serious Adverse Drug Reaction (SADR):
An adverse drug reaction (ADR) is an adverse event (AE) for which a causal relationship to the trial product is at least possible i.e., causal relationship is conceivable and cannot be dismissed. Serious adverse reaction (SAR): Adverse event which fulfils both the criteria for a Serious Adverse Event and the criteria for an Adverse Reaction.

Suspected Unexpected Serious Adverse Reaction (SUSAR):
An SAE which is unexpected and regarded as possibly or probably related to the trial/study product by the investigator.

Medical Events of Special Interest (MESI): A MESI is (1) a medication error (e.g., wrong drug administration or wrong route of administration) or (2) a suspected transmission of an infectious agent via the product.

Non-Serious Adverse Event:
A non-serious AE is any AE which does not fulfil the definition of an SAE.

Severity Assessment Definitions:
• Mild: Transient symptoms, no interference with the subject's daily activities
• Moderate: Marked symptoms, moderate interference with the subject's daily activities
• Severe: Considerable interference with the subject's daily activities, unacceptable

Relationship to study medication Assessment Definitions:
• Probable: Good reasons and sufficient documentation to assume a causal relationship
• Possible: A causal relationship is conceivable and cannot be dismissed
• Unlikely: The event is most likely related to an etiology other than the trial product

Medical Dictionary for Regulatory Activities (MedDRA) will be used for coding adverse events.

Outcome Categories and Definitions:

- Recovered: Fully recovered or by medical or surgical treatment the condition has returned to the level observed at the first trial related activity after the subject signed the informed consent
- Recovering: The condition is improving, and the subject is expected to recover from the event. This term should only be used when the subject has completed the trial.
- Recovered with sequelae: As a result of the AE, the subject suffered persistent and significant disability/incapacity (e.g., became blind, deaf, paralysed). Any AE recovered with sequelae should be rated as an SAE.
- Not recovered
- Fatal
- Unknown

Collection, Recording and Reporting of Adverse Events

All events meeting the definition of an adverse event must be collected and reported from the first trial related activity after the subject has signed the informed consent and until completion of the trial.

Follow-up of Adverse Events

Dr. Backeljauw, the sponsor-investigator, and Cincinnati Children's Hospital Medical Center will provide adequate medical care to the study subject for any study-related adverse events, including clinically significant laboratory values related to the study. This medical care for study subjects will be provided regardless of their insurance status.

All adverse events classified as serious or severe or possibly/probably related to the trial product will be followed until the subject has recovered and all queries have been resolved.

Pregnancy

Subjects will be instructed to notify the sponsor-investigator immediately if they become pregnant. Dr. Backeljauw, the sponsor-investigator, will report to Novo Nordisk any pregnancy occurring during the trial period.

Precautions/Over-dosage

All subjects will receive appropriate education about proper administration of medication from a trained medical professional. The subject's parent will be required to demonstrate proper technique and dosing prior to distribution of medication. They will be asked to notify the study team in case of an overdose and the PI will determine if further medical monitoring is needed based on the described event.

10. COMPENSATION

For each study visit, the study will cover the costs of economy class plane tickets for the primary research subject and for one parent. If the family prefers to drive to CCHMC, the study will reimburse the family at the standard CCHMC mileage reimbursement rate

for the distance from their home address to CCHMC as determined by Google Maps. Additionally, for the initial 12-month trial period the study will cover costs of the hotel rooms for up to 2 nights per study visit as well as the cost of transportation from and to the Cincinnati airport. A meal allowance of $35 per individual per day will be covered. In the extension trial period, the study will cover cost of a hotel room for one night per study visit as well as a meal allowance of $40 per individual per day. There will be no additional compensation for participation the study. The study will cover the costs of all medical evaluations detailed as part of this protocol that occur at CCHMC. GH and supplies will be provided to the subjects for free.

11. STATISTICAL CONSIDERATIONS

This is a pilot single arm study and thus no formal statistical comparison is planned. The outcome measures will all be descriptive. Therefore, we based our power calculations on our ability to confidently detect a positive effect on height SDS. We based the power calculation on our retrospective data. This sample size calculation is based on the following key assumptions: the mean first year response to GH of the 13 previously treated patients in our retrospective data was 0.407 SDS with a standard deviation of 0.343 SDS. We estimated that a sample size of 10 subjects will give us 90% power to demonstrate a change in SDS greater than zero at one-year post treatment initiation. Based on the retrospective data, we are 95% confident that the true mean response falls within the confidence interval of 0.19 to 0.62 SD. This sample size is feasible given the available patient population and estimated budget.

12. DATA HANDLING AND RECORD KEEPING

All data will be collected in standardized case report forms. Forms will be kept in a locked cabinet in a secure location within the Endocrine department. Only the PI and study coordinator will have access to the forms. Review of all CRFs for completion will be done within 1 week after each study visit.

13. ETHICS

Written informed consent will be obtained from all subjects as well as written assent in subjects aged 11 years or older as detailed above. Consent processes will comply with all guidance and regulations of Cincinnati Children's Hospital Medical Center.

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All subjects will be assigned a coded ID and all forms will only contain the coded ID. The code linking the ID with identifiable information will be kept on a password protected secure server as part of the Cincinnati Children's Hospital Medical Center research computing network. Only the PI and approved study personnel will be given access to this code.

The study will be conducted in accordance with the Declaration of Helsinki and will be conducted in accordance with the ICH GCP guidelines. Dr. Backeljauw, the sponsor-investigator, will comply with all applicable regulatory and legal requirements, ICH GCP

guidelines and the Declaration of Helsinki in obtaining and documenting the informed consent.

14. LIABILITY AND SUBJECT INSURANCE

As noted above, during and following a subject's participation in trial, Dr. Backeljauw and Cincinnati Children's Hospital Medical Center will provide adequate medical care to the study subject for any study-related adverse events, including clinically significant laboratory values related to the study. This medical care for study subjects will be provided regardless of their insurance status.

Dr. Backeljauw will be responsible for the conduct of the study and agrees to defend, indemnify, and hold harmless Novo Nordisk, any of its parent companies, affiliates, or subsidiaries, and their respective officers, directors, employees, agents, representatives, distributors, salespersons, customers, licensees, and end-users from and against any claim, suit, demand, loss, damage, expense or liability imposed by any third party arising from or related to: (a) any breach of Dr. Backeljauw's obligations or representations; or (b) Dr. Backeljauw's negligent or grossly negligent use or willful misuse of the study drug, the results, or services derived therefrom. This indemnification shall not apply in the event and to the extent that a court of competent jurisdiction or a duly appointed arbiter determines that such losses or liability arose as a result of Novo Nordisk's gross negligence, intentional misconduct, or material breach of its responsibilities.

15. PUBLICATION PLAN

Dr. Backeljauw intends to publish the results of this trial within 12 months after completion in a peer-reviewed medical journal. He will also present the results of the trial at a national meeting such as the Endocrine Society or Pediatric Endocrine Society annual meetings. This trial is registered on clinicaltrials.gov.
Dr. Dauber is currently a co-investigator. He was involved in the development of the initial study protocol, but not in the development of the extension studies. He does not have access to the raw study data. He will be involved in the development of abstracts/manuscripts that come out of the studies, and therefore will be exposed to deidentified growth and laboratory data from the studies. None of the study funds go towards Dr. Dauber's involvement in the study.

16. STUDY TIMELINE

IRB approval and an IND will be obtained prior to study initiation. We anticipate that it will take 6-18 months to recruit 10 eligible subjects. The study will then take an additional 36 months for the final patient to complete three years of treatment. Eligible subjects that are willing to enroll in the extension trial will come in for their final study visit in 2021 or 2022.

17. STUDY TEAM

Philippe Backeljauw MD, Pediatric Endocrinologist – Principal Investigator
Gajanthan Muthuvel, MD, Pediatric Endocrinology Fellow – Co-Investigator

Leah Tyzinski, BA, CCRP – Study Coordinator
Andrew Dauber MD MMSc, Pediatric Endocrinologist – Co-Investigator Off-site
Hee Kim MD, Radiologist – Co-Investigator
Paul Gubanich MD, Sports Medicine Physician – Co-Investigator
Mark Paterno, MD – Co-Investigator
Jeff Taylor-Haas, PT – Physical Therapist

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