Treatment with recombinant human insulin-like growth factor 1 (rhIGF-1) in patients with pappalysin-2 (PAPP-A2) gene mutation.

1. Background and Study Rationale:

The Cincinnati Center for Growth Disorders (CCGD) diagnosed three siblings (one female and two males) with a novel syndrome of progressive growth failure due to loss of PAPP-A2 function. These patients were identified via homozygosity mapping followed by exome sequencing and were found to have a novel missense mutation in *PAPPA2* (Ala1033Val). In addition, mouse models of PAPP-A2 deficiency have remarkable similarities to our patients in which the animals demonstrated decreased birth weight and postnatal growth retardation[1, 2]. Furthermore, our colleague Jesus Argente, chief of pediatric endocrinology at the Children's Hospital in Madrid, has identified a second family with a homozygous frameshift mutation in *PAPPA2* leading to the exact same clinical and biochemical phenotype as our patients. These are the first two families ever found with mutations in *PAPPA2*.

The three affected patients are offspring of consanguineous parents (first cousins of Middle-Eastern decent). The eldest affected patient is an 18-year old female who has completed her growth. Her adult height is 138.6 cm (4'6"), which is -3.8 SDs below the mean. Her 13- and 9-year old brothers are respectively -2.68 and -2.72 standard deviations below the mean. Additionally, they have two unaffected sisters who are heterozygous for the mutation.

PAPP-A2 (pregnancy-associated plasma protein A2) is a plasma protease member of the papalysin family of metalloproteinases encoded by the PAPP-A2 gene. The protein itself cleaves IGF binding proteins, predominantly IGF binding protein-3 (IGFBP-3) and IGF binding protein-5 (IGFBP-5)[3]. Through this action, PAPP-A2 is believed to be a main regulator of IGF-I bioactivity and therefore indirectly of growth itself, as most of growth hormone's (GH) actions are mediated through IGF-I. The cleavage of IGF-I from its binding proteins frees up the bound form of IGF-I and allows it to become biologically active as free IGF-I. If the protease activity is defective, free IGF-I concentrations will be low, but total IGF-I concentrations will be elevated. As expected, the three patients studied by the CCGD have elevated concentrations of IGF-I and IGFBP-3 and low concentrations of free IGF-1 and decreased IGF bioactivity.

The two youngest affected males are approaching or are in early puberty and the remaining time for further growth is limited. We hypothesize that an alternative approach to improve their growth could be treating them with recombinant human IGF-1 (rhIGF-1, Increlex®), which is FDA-approved in the United States for the treatment of severe primary IGF-1 deficiency. Patients with severe primary IGF-I deficiency often have GH resistance due to a defect in the GH receptor. These patients also have low free IGF-I concentrations. Long-term treatment with recombinant human IGF-1 has shown to improve these patients' adult height. Therefore, we hypothesize that the administration of recombinant human IGF-1 to patients with PAPP-A2 mutation will result in an increase in the levels of free IGF-1 concentration and hence increase in linear growth.

2. Hypothesis and Specific Aims.

General Hypothesis

Treatment with subcutaneous injection of recombinant human IGF-1 in patients with short stature secondary to PAPP-A2 deficiency will increase height velocity.

Specific Aims

a. Determine the pharmacokinetic parameters of a single SC injection of rhIGF-1 concentration in patients with the PAPP-A2 mutation.
b. Describe the effects of one year of treatment with rhIGF-1 administered via subcutaneous injection in patients with PAPP-A2 mutation. If the treatment is well-tolerated, treatment will continue until adult height is achieved.
c. Assess the PK/PD relationship (PD marker being IGFBP-3) during the one year of treatment
d. Describe additional phenotypic features of patients with PAPP-A2 deficiency including glucose handling, body composition, bone geometry and bone density.
e. Monitor and quantify the potential development of antibodies to administered rhIGF-1 over a period of 1 year treatment.

3. Preliminary Results.

Treatment with rhIGF-1: Our colleague Jesus Argente from Madrid, Spain is currently executing a similar clinical trial in which two young siblings (male and female) with PAPP-A2 mutation have been receiving twice daily SC rhIGF-1 treatment. The preliminary results show a favorable response in linear growth of both affected individuals.

Pharmacokinetic study: Dr. Wolfgang Högler and colleagues have recently published a well-designed pharmacokinetic study that describes free and total IGF-1 response to a single dose of rhIGF-1 in patients with IGF acid labile subunit (ALS) deficiency caused by IGFALS mutations[4]. These patients have similar physiology to patients with PAPP-A2 mutations as ALS prolongs the half–life of circulating IGF-1, and mutations in *IGFALS* result in decreased free IGF-1 levels. However, there are important distinctions between the two conditions due to the different pathophysiological mechanisms. As part of our study, we intend to mimic their protocol by applying it to patients with a PAPP-A2 mutation, which will allow for future comparison of our results to those of the ALS patients. We are already collaborating with Dr. Jan Frystyk, one of the co-authors on the ALS manuscript, who performed all of the free IGF-1 assays.

4. Study Design and Methods.

Our study is divided in to three different parts:

- 24-hour pharmacokinetic response of free and total IGF-1 and IGF binding protein-3 (IGFBP-3) to a single dose of rhIGF-1 (120 mcg/kg) in three patients with PAPP-A2 mutation compared to up to four unaffected heterozygous relatives and 2 healthy adult controls. This part is complete.

- One-year trial of rhIGF-1 at standard dose given to the two youngest males with PAPP-A2 mutation. The primary end point of this trial will be first year height velocity. Secondary outcomes will include change in height SDS, change in height velocity SDS, and change in whole body and lumbar spine bone mineral density. This part is complete, and will be followed by an extension part. Provided continued improved height velocity (defined as height velocity at the 25th percentile or better) without adverse effects, treatment with IGF-1 will be continued on an annual basis until closure of the growth plates indicating achievement of near-adult or adult height (bone age ≥ 15 years or 17 years, respectively, for males).

- Description of additional phenotypic characteristics of patients with PAPP-A2 mutation by studying glucose and insulin metabolism, body composition, bone geometry and bone density before and after treatment with rhIGF-1. This part will be complete in 2019 for the subjects not on Increlex.

4.1. PK response to rhIGF-1:

The primary objective of this study is to determine the pharmacokinetic (PK) effect of a single injection of rhIGF-1 in patients with PAPP-A2 mutations compared to heterozygous carriers and healthy controls.

Subject Recruitment: This is an n=7 study. A total of seven individuals will be recruited to participate in this study. Among the participants, we will have the three affected siblings with PAPP-A2 mutation (two males and one female), their heterozygous relatives (2 parents) and 2 healthy adult controls. We will have a thorough conversation with them about the risks and benefits of participating in this study. Written informed consent will be obtained. The affected subjects and family members will all be recruited directly by Dr. Andrew Dauber. They have already participated in the initial research protocol which identified the genetic defect. Each family member will be allowed to decide for themselves whether they wish to participate and it will not be necessary for all family members to participate in order to proceed with the study. All travel costs and accommodations will be covered by the Cincinnati Center for Growth Disorders. No additional compensation will be given to the subjects or their family members.

Recruitment of controls: 2 healthy adult controls (one male and one female) between the ages of 18-30 will be recruited from community and/or university advertising. Additionally, one of the co-investigators who meets all eligibility criteria will act as the female control subject. In order to be included in our study, the volunteers will have to be between the ages of 18-30, be in good general health and not taking any medications (with the exception of contraceptives). The candidates will have an initial screening visit in the outpatient CTRC where they will undergo full physical examination. Additionally, the following fasting labs will be obtained: CBC, renal profile, lipid profile, HgbA1C, insulin, IGF-1, IGFBP-3, random growth hormone, and urine pregnancy test (which will be repeated prior to starting the study). Any abnormal lab results or physical exam finding will result in exclusion from the study. The subjects will be informed of any abnormal lab results. They will be contacted via telephone and will receive a copy of the lab results. In addition, they will be referred to their PCP for further evaluation and treatment (if needed). We will have a thorough conversation with them about the risks of participating in this study. Written informed consent will be obtained. Volunteers will be paid $500 upon completion of all study procedures.

Study Protocol: The patients, their heterozygous relatives and the healthy controls will come to the outpatient clinical translational research center (CTRC) on the morning of the study. They will be told to fast after midnight the prior evening. An indwelling venous catheter will be placed. A urine pregnancy test will be obtained from all females participating in our study. Baseline levels for IGF-1, free IGF-1, IGFBP-3, IGF-1 bioactivity, GH and blood glucose will be obtained between 8-9 AM. At 9:00 h a single injection of rhIGF-1 (mecasermin, Increlex®) will be administered at a dose of 120 mcg/kg. This dose was chosen in order to be able to compare to the study performed by Dr. Högler as mentioned in the preliminary data. Breakfast will be provided immediately after the SC injection of rhIGF-1. Blood samples for GH, IGFBP-3, IGF-1, free IGF-1, IGF-1 bioactivity and blood glucose will be repeated every 30 minutes, then every hour, every two hours until 9 PM. Subjects will then be sent home and a final sample will be drawn at 9 AM the following morning (a 24 hour time point) as detailed below. Approximately 5 ml of whole blood will be drawn per sample. The total amount of blood for this study is less than 5% of the blood volume for the youngest subject. Additionally, blood glucose monitoring (by finger stick glucose measurement) will be available as needed for symptoms of hypoglycemia.

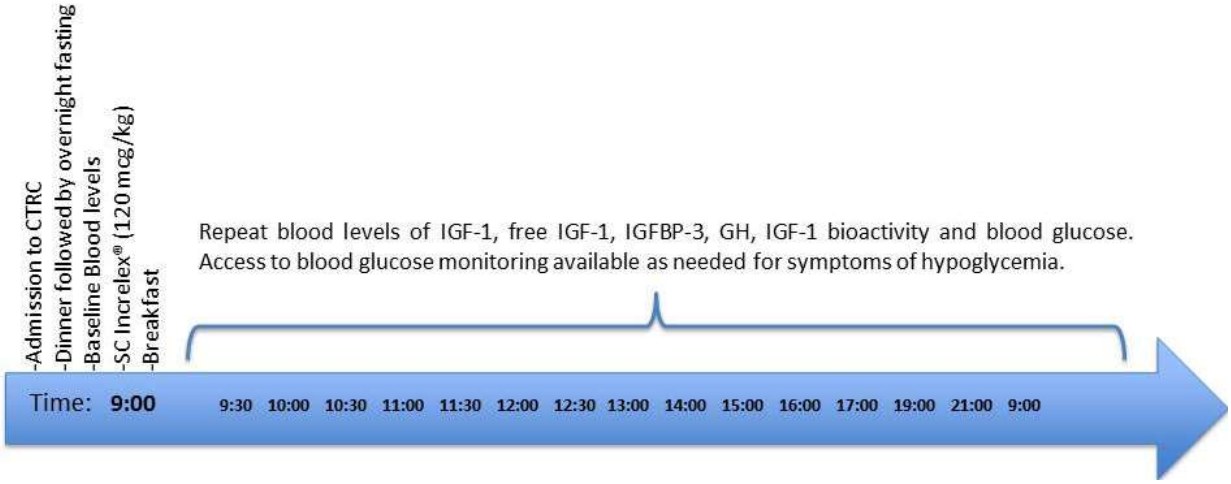


Figure 1

4.2. <u>Prolonged rhIGF-1 treatment:</u>

This phase of the study, will serve as a proof of principle to demonstrate that subcutaneous administration (via 2 injections daily) of rhIGF-1 is effective in improving the growth velocity in patients with PAPP-A2 mutation. The FDA-approved dosing regimen will be applied to these patients during the entire treatment period.

Subject recruitment: This is an n=2 study. The two youngest male siblings with PAPP-A2 mutation will participate in this protocol. We will have a thorough conversation with them and with their parents about the risks and benefits of participating in this study.

Study Protocol: Our patients will be given twice daily SC injections of rhIGF-1 (Mecasermin, Increlex®) longitudinally first for a period of one year. To assess the effectiveness of the treatment, the height velocity in our patients will be compared to their pre-treatment data which is available from our previous

measurements. The patients will come to the CCHMC CTRC for a series of outpatient research visits. The lab and assessment schedule is detailed below.

On day 1, the patients will present to the outpatient CTRC. They will undergo a full physical examination with close attention to pubertal staging. ECG will be performed. Baseline height in triplicate (cm) and weight (Kg) will be measured using a wall-mounted Harpenden stadiometer and electronic scale respectively. During this visit, fasting blood samples will be obtained in addition to further testing including DXA scan, pQCT and US as detailed in section 4.3.

Following the initial PK study at CCHMC, the patients will then undergo a one-year treatment trial with rhIGF-1 which will be started at 60 mcg/kg BID SC given twice daily for one week. If well tolerated, the IGF-I will be increased to 120 mcg/kg BID SC and the patients will be maintained at this dosage for one year. Doses will be given within 20 minutes of eating breakfast and dinner. Tolerability will be determined with frequent blood glucose measurements via finger stick. The patients will be instructed to measure their blood glucose 3 times per day: fasting, between 10:00-12:00 h, and between 20:00-22:00 h. In addition, they will keep a daily log which we will review via telephone or e-mail. Once the dose is increased to 120 mcg BID, they will continue to measure their blood glucose for one additional week. If no significant episodes of hypoglycemia occur, then they can continue to measure their blood glucose only as needed for symptoms of hypoglycemia. If hypoglycemia dose occur, the parents will be instructed to treat the patient with fast acting carbohydrates (such as juice) and to recheck the blood sugar in 15 minutes as we do with our patients with type 1 diabetes.

They will be re-evaluated as outpatients after 3 months, 6 months, 9 months and 12 months of treatment for serial height measurements and for close monitoring of possible side effects. The dose of rhIGF-I will be adjusted for weight at each of these visits. ECG will be performed at baseline and at each of the study visits. Echocardiogram will be performed at the 6 month and 12 month visit. A funduscopic, oropharyngeal, back and hip exam will be performed at baseline and during each of the study visits. A glucometer and testing strips will be provided for home monitoring of blood glucose concentration. We will have monthly follow up calls with the family to assess for any complications. In addition, we will be in close contact with the patient's primary care provider and local endocrinologist who will receive a detailed letter after each of the follow up visits. If any unexpected issues arise we will help coordinate prompt follow up with PCP or with local endocrinologist.

Study drug is being donated by Ipsen (the drug manufacturer) and will be provided to the patients by the study team at no cost.

Dosage Interruption – In the event that either participant experiences a dosage interruption of >48 consecutive hours during the one year trial, the participant will be restarted at a dose of 60 mcg/kg BID SC given twice daily for one week. If well tolerated, the IGF-I will be increased to 120 mcg/kg BID SC and the patient will be maintained at this dosage for the remainder of the one year trial.

4.3. Additional phenotypic description of patients with PAPP-A2 mutation.

This part of our study will investigate further phenotypic characteristics of patients with *PAPPA2* mutations including glucose handling, body composition, bone geometry and density and renal/spleen size.

Subject recruitment: This is an n=3 study. We will perform this testing on all 3 affected siblings at baseline. We will then repeat this testing in the two siblings who are undergoing IGF-1 therapy at the 1 year time point. If either of the siblings stops the IGF-1 therapy prior to the completion of the 1 year trial, the below testing (as shown in table 1 and figure 2 below for 12 months) will still be completed at the 1 year time point for both siblings.

Study protocol: In order to achieve this, the following tests will be performed in all three siblings affected by the PAPP-A2 mutation:

- **Dual energy-x-ray absorptiometry (DXA):** bone mineral content (BMC), bone mineral density (BMD), fat-free mass (FFM), and estimates of percent body fat will be measured using DXA scans of total body, lumbar spine, hip and forearm. DXA scans will be acquired using the Hologic Discovery and standard positioning as described by the manufacturer. Scans will be analyzed using software Apex 4.0. To avoid the size-related DXA artifacts typical of individuals with short stature, age- and gender-specific results will be corrected for body size.
- **Peripheral quantitative computed tomography (pQCT):** pQCT scans will be obtained on the tibia at the distal 3%, 38% and 65% sites. The tibia at the 3% site is within the distal metaphysis and is composed primarily of trabecular bone. The tibia at the 38% site is located in the diaphysis and is predominantly composed of cortical bone. Additionally, the tibia at the 65% site provides information about the relationship between bone and muscle mass. pQCT scans will be acquired using the Stratec XCT 2000 scanner with a voxel size of 0.4 mm and a speed of 25 mm/sec. Scans will be analyzed with software version 5.50. Outcome variables analyzed will include trabecular and total volumetric BMD at the distal radius as well as cortical density and thickness, BMC, muscle area and the ratios BMC to muscle and cortical to total cross-sectional area at the distal radius.
- **Bone age:** Standard left hand and wrist X rays will be obtained to asses bone age using the Greulich–Pyle Atlas.
- **Renal and spleen ultrasound:** IGF-1 plays an important role in organ growth and some patients treated with IGF-1 therapy have been noted to have more rapid growth of the kidneys and spleen, especially during the first 2-3 years of their therapy
- **Oral glucose tolerance test**
- **Potential development of antibodies to administered rhIGF-1**

Table 1

Lab and assessment schedule:

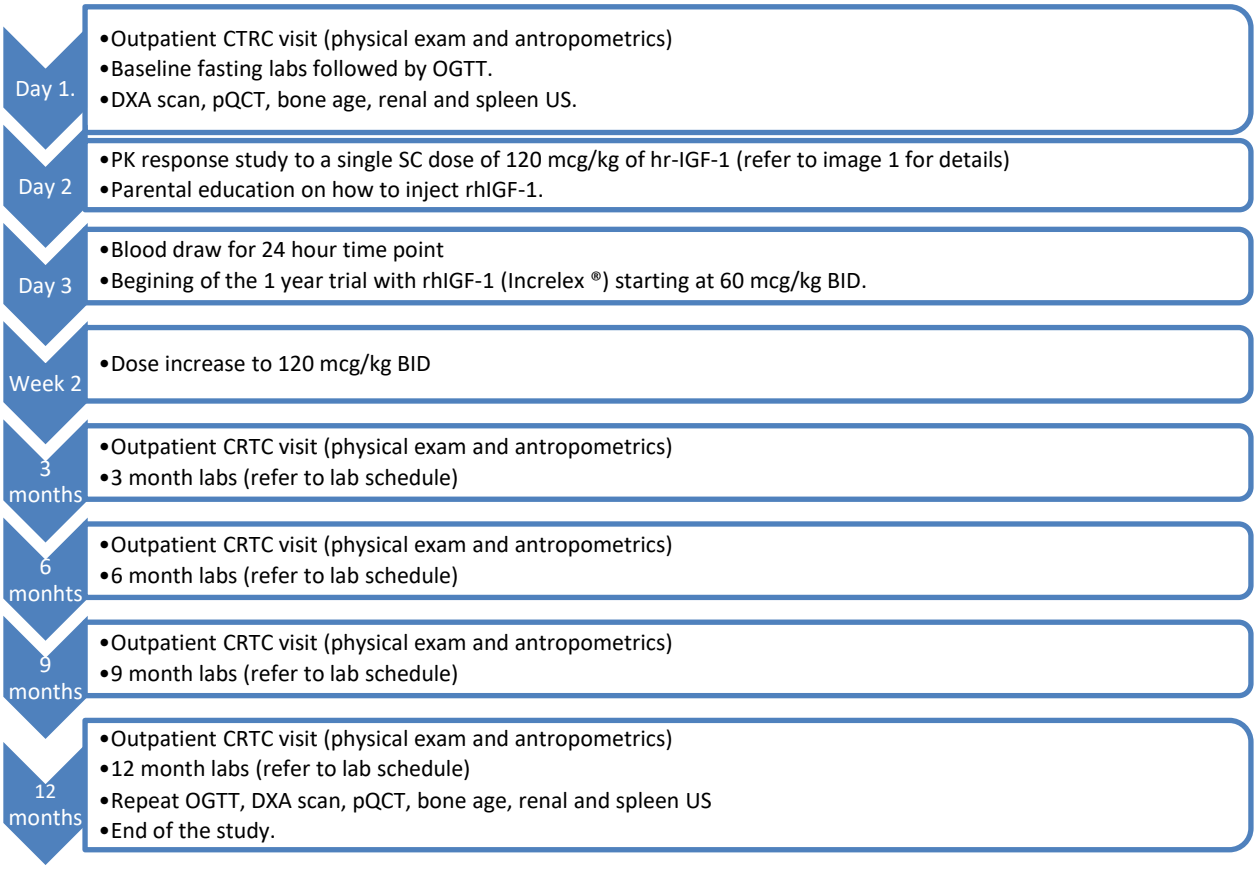
Lab	Day 1	3 Months	6 Months	9 Months	12 Months
CBC	X				X
Metabolic Panel*	X	X	X	X	X
Lipid Profile	X				X
OGTT	X				X
A1C	X		X		X
Insulin	X	X	X	X	X
Glucose	X	X	X	X	X
GH	X	X	X	X	X
IGF-I	X	X	X	X	X
IGFBP-3	X	X	X	X	X
ALS	X	X	X	X	X
Free IGF-I	X	X	X	X	X
PAPP A2	X	X	X	X	X
rhIGF-I Antibodies	X	X	X	X	X
Osteocalcin					X*
C-telopeptide					X*
Additional Testing:					
DXA scan	X				X*
ECG	X	X	X	X	X
Echocardiogram			X		X
pQCT	X				X*
Bone age	X				X*
Renal US	X				X*
Spleen US	X				X*

***Comprehensive Metabolic panel includes: Electrolytes, BUN, Creatinine, Calcium, Glucose, Albumin, Total Protein, AST, ALT, ALP (Alk Phos), and Total Bilirubin**

*This testing will only be repeated in the patients with PAPP-A2 mutation who received rhIGF-1 treatment.

Timeline of the study:

Figure 2



5. Available and Needed Resources

Investigators will use the CTRC for admitting and following up the patients. The CTRC core laboratory and the endocrinology laboratory will be used to measure most of the laboratory parameters noted above. Some specialized samples including free IGF-1 and IGF-1 bioactivity will be analyzed either in Dr. Hwa's or in one of our collaborator's laboratory.

6. Investigators (Initial Trial):

Philippe Backeljauw, MD (Principal Investigator), Jacob Redel, MD (Co-Principal Investigator), Leah Tyzinski (Coordinator for the CCGD), Catalina Cabrera Salcedo MD (Fellow), Vivian Hwa (Co-investigator), Melissa Andrew (Lab technician), Halley Wasserman (Fellow).

7. Statistical Analysis

The one-year trial with rhIGGF-1 in two patients with PAPP-A2 mutation is a descriptive study which will not have a formal statistical analysis. We will describe the changes in growth velocity, height SDS, bone mineral density and other biochemical parameters, but formal statistical analysis will not be performed.

A non-compartmental analysis will be performed on the total IGF-1 concentrations and free IGF-1 concentrations to assess the PK parameters: C_{max}, t_{max}, $AUC_{0\text{-}24h}$, and $AUC_{0\text{-}12h}$ with and without IGF-1 baseline correction. A non-compartmental analysis will be also performed on the IGFBP-3 concentrations to assess the following parameters: C_{max}, t_{max}, $AUC_{0\text{-}24h}$, , and $AUC_{0\text{-}12h}$ with and without IGFBP-3 baseline correction. All results will be presented as summary measures with confidence intervals as well as graphical displays for the affected individuals, the heterozygous relatives and the two healthy controls as three separate groups.

8. Study Consents

This study is designed for one specific family as described above. We have been in contact with the patients and their parents because of the prior study in which one of the siblings participated. The family is very interested in participating in our study protocol. Because the family lives in New York, Dr. Dauber will have telephone contact with the research subjects, to be able to review all of the details of the protocol including the risks and benefits. Separate phone calls will be made with each of the adult siblings. The potential of not benefiting from this study will also be discussed. The family will be forwarded a copy of the consent form in advance of these conversations. The family will be given a full opportunity to ask any questions. The family will also be asked to repeat back to the investigators their understanding of the study. In addition, verbal and written assent will be obtained from the two minors enrolled in the study. If the family continues to be interested, we will then schedule commencement of the treatment at CCHMC. This entire process will then be repeated face to face, in person, upon arrival to CCHMC. If the family continues to agree to their participation, informed consent documents will be signed. Travel costs to CCHMC for the initial study and follow-up visits will be paid for. The investigators will completely ensure that participation is voluntary. The investigators will collaborate with the primary care physician and local endocrinologist when the family returns home in-between visits to CCHMC.

9. Risks/Benefits.

The main benefit expected from this study is to improve the linear growth of the two young males affected by the PAPP-A2 mutation. In addition, this study will contribute to a better understanding of the recently described PAPP-A2 mutation and potential co-morbidities as well as treatment options for affected individuals.

RhIGF-1 side effects: The major risks from this trial are related to the treatment with rhIGF-1 (Increlex®) itself. Known side effects of this FDA-approved medication include hypoglycemia, hypersensitivity (allergic reactions), intracranial hypertension, lymphoid tissue hypertrophy (which can include tonsillar hypertrophy), slipped capital femoral epiphysis (SCFE) and progression of scoliosis, echocardiographic evidence of cardiomegaly/valvulopathy without associated symptoms, liver

dysfunction, alopecia, osteonecrosis, systemic hypersensitivity, and potential initiation and progression of malignant tumors. Our patients will receive their initial dose of rhIGF-1 in a controlled setting and they will be closely monitored for the development of any of these adverse effects.

- Hypoglycemia: The patients will receive a glucometer for blood glucose monitoring t home. They will be instructed to measure their blood glucose regularly during the first 2 weeks of treatment. If no significant episodes of hypoglycemia occur, they can continue to measure their blood glucose only as needed for signs or symptoms of hypoglycemia.
- 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 in order to rule out papilledema.
- Lymphoid hypertrophy: Tonsillar hypertrophy will be assessed clinically at baseline and at each follow up visit.
- 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 in order to rule out SCFE.
- Cardiomegaly/valvulopathy: ECG will be done at baseline and all study visits; echocardiogram will be performed at 6 months and 12 months visits
- Liver dysfunction: a comprehensive metabolic panel will be performed at baseline and each study visit
- Development of malignant neoplasia: If malignant neoplasia develops in any patients receiving Increlex therapy, treatment will be immediately discontinued and the patient will be referred for the appropriate medical care.

In addition, we will be in close contact with the patient's primary care provider and local endocrinologist who will receive a detailed letter after each of the follow up visits. If any unexpected issues arise we will help coordinate prompt follow up with PCP or with local endocrinologist.

Injection site reactions: The patients can experience local symptoms related to the subcutaneous administration of rhIGF-1 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).

Immunogenicity: There is a risk of potential development of antibodies to administered rhIGF-1. Blood samples will be taken at baseline, periodically and at the end of the study to monitor the potential generation of antibodies.

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[5, 6]:

- DXA scan (total body, lumbar spine, hip and forearm): 0.013 mSv; two DXA scans: 0.027 mSv
- peripheral QCT: less than 0.01 mSv; two peripheral QCT: less than 0.02 mSv
- Bone age (X –ray of the hand): 0.005 mSv; two bone age studies: 0.01 mSv

Therefore, total radiation exposure from this study is expected to be 0.05 mSv or less (equivalent to the amount an individual receives over 6 days from background radiation).

10. Reasons for participant withdrawal from the one year clinical trial

If any of the male patients develop any of the following adverse events, the patient will be withdrawn from the one year trial:

- Severe hypoglycemia defined as blood glucose of less than 50 on more than one occasion
- Diagnosis of intracranial hypertension
- Diagnosis of SCFE (slipped capital femoral epiphysis)
- Diagnosis of malignant neoplasia

If withdrawn from the 1 year trial treatment, the patient will remain in the phenotypic data collection aspect of the trial, and measures as described in Table 1 and Figure 2 for the 12 month/1 year time point will be conducted as scheduled.

11. IND Status

This study will be performed under an IND from the FDA.

ADDENDUM:

Extension of the treatment arm of the clinical trial

1. Background and preliminary results:

With the objective to improve the growth of our patients affected by progressive growth failure due to a homozygous loss-of function mutation in the PAPP-A2 gene (Ala1033Val), we are currently implementing the pilot interventional study that aims to describe the effects of one year of treatment with recombinant human IGF-1 (rhIGF-1, Increlex®) on growth in patients with this specific condition as well as to characterize the progression of the patients' phenotype over time. We hypothesized that rhIGF-1 would increase the concentrations of free IGF-1 and hence increase linear growth.

The two youngest affected males were started on treatment with rhIGF-1 therapy at a dose of 60 mcg/kg which was subsequently increased to a full dose of 120 mcg/kg in December of 2015. Unfortunately, the eldest brother developed pseudotumor cerebri (which is a known side effect of Increlex®) on day of treatment 50 which resulted in therapy discontinuation. The youngest brother was also temporarily suspended from treatment for 54 days due to regulatory concerns. This event was reported to the IRB, the FDA and IPSEN (study sponsor) in a timely manner. All the parties agreed to

continue treatment on the youngest subject, and therefore he was restarted on treatment as detailed in the IRB protocol. Neither patient experienced hypoglycemia nor any other serious adverse events.

The youngest patient has now been on treatment for 36 months and he is tolerating the medication well. Thus far, the response to therapy is encouraging; his pre-treatment height velocity was 3 cm/year, and his post-treatment height velocity is currently 5.2 cm/year. Furthermore, our colleague Jesus Argente, chief of pediatric endocrinology at the Children's Hospital in Madrid, recently published his experience in treating two pre-pubertal siblings from another family also affected by a homozygous PAPP-A2 gene mutation (p.D643fs25*). Treatment with rhIGF-I accelerated growth velocity to an average of up to 7.3 cm/yr, and improved their height SDS. No hypoglycaemia or any other side effects were reported during treatment in these two patients[7].

2. Main Objective and secondary objectives:

Main objective:

- To provide ongoing treatment with rhIGF-1 (Increlex®) to our patient who has benefited from previous treatment (as detailed in the preliminary results section) and to closely monitor his continued response to treatment.

Secondary objectives:

- Assess the long-term safety and tolerability of rhIGF-1 in one subject with PAPP-A2 gene mutation
- Describe the evolution of several metabolic parameters including glucose handling, body composition, and bone mineral density in the three affected siblings with PAPPA2 gene mutation.

3. Study design and methods:

Subject recruitment: No additional participants will be recruited for this extension protocol.

2. Study protocol:

This is an extension trial of rhIGF-1 at a standard dose given to one subject (youngest affected male) with PAPP-A2 gene mutation who has benefited from previous treatment. The primary outcome measures of interest are height velocity and change in height standard deviation scores (Ht SDS). Our patient will continue to receive twice daily SC injections with rhIGF-1 (Increlex®) at a standard dose of 120 mcg/kg/dose. Additionally, there will be ongoing phenotyping which will be performed in all affected siblings at the yearly study visits.

The parents have been previously trained on glucometer use which they have available to be used as needed for signs and symptoms of hypoglycemia. If hypoglycemia does occur, the parents have been instructed to treat the patient with fast acting carbohydrates (such as juice) and recheck the blood glucose in 15 minutes.

For monitoring, the patient will come to the CCHMC CTRC for a series of outpatient research visits every 4 months during the second year of treatment, and every 6 months for subsequent years of treatment. The lab and assessment schedule is detailed below. At each study visit, we will obtain a height in triplicate (cm) and weight (kg) measured using a wall-mounted Harpenden stadiometer and electronic scale respectively. Additionally, fasting blood samples will be obtained. The patient will be evaluated as an outpatient at 16 months, 20 months, 24 months and every 6 months thereafter for serial height measurements and for monitoring of possible side effects. Each visit has a +/- 2 week window to allow for travel schedules. The dose of rhIGF-I will be adjusted for weight at each of these visits. A funduscopic, oropharyngeal, back and hip exam will be performed during each of the study visits. The family will be instructed to call us in-between study visits should any adverse reactions occur. In addition, we will be in close contact with the patient's primary care provider and local endocrinologist who will receive a detailed letter after each of the follow up visits. If any unexpected issues arise we will help coordinate prompt follow up with PCP or with local endocrinologist. Study drug is being donated by Ipsen (the drug manufacturer) and will be provided to the patients by the study team at no cost.

The three affected siblings (two males, one female), will be assessed at the yearly outpatient study visits for further phenotyping. During this visit, we will obtain fasting blood samples in addition to the following tests:

- Oral glucose tolerance test (OGTT)
- Dual energy-x-ray absorptiometry (DXA) scan of whole body, lumbar spine, hip and forearm.
- The two affected brothers that are still growing will get a repeat bone age x-ray.
- The affected younger brother on rhIGF-I treatment will have an electrocardiogram, and repeat renal and spleen ultrasounds. (Please refer to the original protocol for full details). These tests were all done at baseline and at the end of the first year of the trial as well. Since this has already been approved, we have not included it in the extension trial application or protocol.

Lab and assessment schedule:

Lab	16 month	20 month	24 month	Monitoring (30, 42, 54 etc. month)	Yearly Phenotyping (36, 48, 60 etc. month)
	Year 2 of Treatment			Year 3+ of Treatment	
CBC			X		X
Metabolic Panel	X	X	X	X	X
Lipid Profile			X		X
Whole Blood			X		X
A1C	X	x	X	X	X
Insulin	X	X	X	X	X
Glucose	X	X	X	X	X
GH	X	X	X	X	X
IGF-I	X	X	X	X	X
IGFBP-3	X	X	X	X	X

ALS	X	X	X	X	X
Free IGF-I	X	X	X	X	X
PAPP A2	X	X	X	X	X
rhIGF-I Antibodies	X	X	X**	X	X**
Osteocalcin			X		X
C-telopeptide			X		X
Vitamin D			X		X
Phosphorus			X		X
Free fatty acids			X		X
Additional Testing:					
ECG			X**		X**
OGTT			X		X
DXA scan			X		X
Bone age			X*		X*
Renal US			X**		X**
Spleen US			X**		X**

*This testing will be obtained on participants that are still growing
** This testing will be obtained only for participants on rhIGF-I treatment

4. Available and needed resources:

Investigators will use the CTRC for all research visits. The CTRC core laboratory and the endocrinology laboratory will be used to measure most of the laboratory parameters noted above. Some specialized samples including free IGF-1 and IGF-1 bioactivity will be analyzed either in Dr. Hwa's or in one of our collaborator's laboratory.

5. Compensation:

We will pay for the cost of travel, hotel and food for the research subjects and one parent. No additional compensation will be provided. This is the same as what was done during the first year of the study.

6. Investigators (Extension trial):

Philippe Backeljauw, MD (Principal Investigator), Jacob Redel, MD (Co-Principal Investigator), Leah Tyzinski (Coordinator for the CCGD), Eirene Alexandrou, MD (Fellow), Andrew Dauber (Co-Investigator), Vivian Hwa (Co-investigator).

7. Study consents: An additional written extension consent and assent will be obtained. See separate document.

8. Risks/ Benefits: These are unchanged.

9. Reasons for premature termination of the clinical trial: These are unchanged.

10. **Reasons for completion of the clinical trial:** When it has become clear that the participant receiving rhIGF1 therapy has finished growing, the treatment arm of the study will be stopped. This is defined by either of the following:
    - The participant reaching full skeletal maturity with a bone age of 17 years, or ≥15.5 years, and:
    - The participant's height velocity is <2cm per year

Once the decision has been made to discontinue treatment, all three affected siblings will be asked to return for one more phenotyping visit a year (+/- 2 weeks) from the date that treatment was stopped. The study labs and assessments will be the same as previous phenotyping visits with the exception of those that are completed as a result of rhIGF1 exposure (i.e. antibody, ECG, renal/spleen ultrasound).

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