

# PAPPA2 as a Therapeutic Modulator of IGF-I Bioavailability: *in Vivo* and *in Vitro* Evidence

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**Context:** Pregnancy-associated plasma protein A2 (PAPPA2) is a protease that cleaves IGF-binding protein (IGFBP)-3 and IGFBP-5, liberating free IGF-I. Five patients from two families with genetic mutations in *PAPPA2* presented with growth retardation, elevated total IGF-I, and IGFBP-3 but decreased free IGF-I.

**Objective:** To determine whether plasma transfusion or recombinant human (rh)PAPPA2 could increase free IGF-I in patients with PAPPA2 deficiency or idiopathic short stature (ISS).

**Design:** Single patient interventional study combined with *in vitro* experimentation.

**Setting:** Academic medical center.

**Patients:** Three siblings with PAPPA2 deficiency and four patients with ISS.

**Interventions:** An adult female with PAPPA2 deficiency received a 20 mL/kg plasma transfusion. PAPPA2, intact IGFBP-3, and free and total IGF-I levels were monitored during 2 weeks. rhPAPPA2 was added to serum from patients with PAPPA2 deficiency and ISS *in vitro* for 4 hours. Intact IGFBP-3 and free IGF-I levels were assayed via ELISA.

**Main Outcome Measures:** Free IGF-I concentrations.

**Results:** Plasma transfusion resulted in a 2.5-fold increase of free IGF-I levels on day 1 posttransfusion with a return to baseline during a 2-week period. *In vitro* studies demonstrated a dose-dependent increase in free IGF-I and decrease in intact IGFBP-3 after the addition of rhPAPPA2. The increase in free IGF-I was more pronounced in patients with PAPPA2 deficiency compared with those with ISS.

**Conclusions:** PAPPA2 plays a key role in regulation of IGF-I bioavailability. rhPAPPA2 is a promising therapy to increase free IGF-I levels both in patients with PAPPA2 deficiency as well as in patients with ISS.

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**Freeform/Key Words:** IGF-I, PAPPA2, short stature

Abbreviations: ALS, acid labile subunit; CCHMC, Cincinnati Children's Hospital Medical Center; FFP, fresh-frozen plasma; IGFBP, IGF-binding protein; ISS, idiopathic short stature; P, patient; PAPPA2, pregnancy-associated plasma protein A2; rh, recombinant human.

The GH/IGF-I axis plays an essential role in human growth. Multiple genetic defects have been found throughout this axis that lead to significantly impaired growth, associated with IGF-I deficiency or IGF-I resistance [1]. IGF-I is the major mediator of GH action and is produced by the liver as well as locally in the growth plate. IGF-I circulates in ternary complex with IGF-binding proteins (IGFBPs) and the 85-kDa acid labile subunit (ALS). The binding of IGF-I to IGFBPs is requisite for association with ALS, as neither IGF-I nor IGFBP can independently associate with ALS [2, 3]. Of the six IGFBPs [4], IGFBP-3 is the main binding protein found in the ternary complexes, although IGFBP-5 also readily associates with both IGF-I and ALS [5]. Although the primary purpose of the ternary complex has been posited to increase the half-life of IGF-I in circulation, these binding proteins may also play an important role in the regulation of IGF-I bioavailability [6].

Levels of unbound, free IGF-I available for biological actions have been investigated in a number of populations, including children [7–10]. It is estimated that free IGF-I represents between 0.4% and 2% of total IGF-I in the serum [7, 8, 10]. Free IGF-I levels increase throughout childhood and peak in puberty, after which the levels fall [8–10]. Diagnostically, free IGF-I levels appear to be a sensitive indicator of GH deficiency but do not provide much additional information in the diagnostic workup beyond that of total IGF-I levels [8–10]. In one study of 16 prepubertal children treated with GH for GH deficiency, free IGF-I levels rapidly increased after the initiation of therapy and to a greater extent than did either total IGF-I or IGFBP-3 levels [10]. Interestingly, the percentage increase in free IGF-I levels after 1 month of treatment was a significant predictor of the increase in height velocity, whereas the increase in either total IGF-I or IGFBP-3 levels had no significant correlation. Taken together with their finding that peak free IGF-I levels correspond to the age of peak height velocity during puberty, the authors suggested that free IGF-I is a key biological mediator of GH action [10]. In another study involving 63 children treated with GH, the molar ratio of IGF-I to IGFBP-3, a proxy for free IGF-I levels, was significantly increased in children with an adequate response to GH therapy compared with nonresponders. These studies, albeit limited, support the hypothesis that increasing free IGF-I levels should lead to increased growth [11].

Pregnancy-associated plasma protein A2 (PAPPA2) is a metalloproteinase that specifically cleaves IGFBP-3 and IGFBP-5, thereby liberating IGF-I from its ternary complex, making it available for binding to cell surface IGF-I receptors and initiating growth-promoting activities [12]. Pappa2 knockout mice have postnatal growth retardation with elevated levels of total IGF-I and low levels of free IGF-I [13, 14]. Recently, the first human patients with genetic defects in *PAPPA2* were reported [14]. Similar to the mouse model, the five affected children from two unrelated families presented with postnatal growth retardation and elevated levels of total IGF-I and IGFBPs, but low levels of free IGF-I and serum IGF bioactivity. The two identified homozygous mutations were shown to abrogate PAPPA2 protease function, resulting in a significant decrease in IGF-I bioavailability despite markedly increased total IGF-I levels [14]. These patients provide convincing human genetic evidence that PAPPA2 plays an important role in regulating IGF-I bioavailability and that defects in its function can affect human growth. Further human genetic evidence comes from large genome-wide association studies in which both *PAPPA2* and its related gene, *PAPPA*, were identified to have nearby common genetic variants associated with adult stature in the general population [15]. Additionally, a more recent genome-wide association study found that rare damaging missense variants in stanniocalcin 2, an inhibitor of PAPPA and PAPPA2 [16], are associated with increased adult height [17]. It is thought that a lack of stanniocalcin 2 activity should lead to increased PAPPA and PAPPA2 activity, resulting in increased IGF-I bioavailability and thus increased growth.

Based on these insights, we sought to provide proof of principle evidence that PAPPA2 could serve as a novel growth-promoting therapeutic agent both in patients with PAPPA2 deficiency as well as in patients with idiopathic short stature (ISS). To achieve this goal, we took two complementary approaches. First, we performed a plasma transfusion treatment in a single adult patient with PAPPA2 deficiency to assess the biochemical response of PAPPA2 replacement therapy *in vivo*. Second, we performed *in vitro* studies in which we combined

recombinant human (rh)PAPPA2 with serum from three patients with PAPPA2 deficiency as well as four patients with ISS and evaluated impact on integrity of IGF-BPs and free IGF-I levels.

## 1. Materials and Methods

### A. Subjects

Three patients (P1, female; P2 and P3, males) with a homozygous p.Ala1033Val missense mutation in *PAPPA2* were included in this study. Details of their clinical presentation have been previously reported [14, 18]. We have previously demonstrated that this mutation leads to a PAPPA2 protein that is expressed at lower levels than wild-type and lacks any proteolytic activity [14]. Written informed consent was obtained from P1 and from the parents of P2 and P3, and written assent was obtained from P2 and P3. All procedures were performed as part of a research protocol approved by the Cincinnati Children's Hospital Medical Center (CCHMC) Institutional Review Board. P1, P2, and P3 had blood drawn for serum isolation and subsequent *in vitro* rhPAPPA2 enzyme activity experiments. The serum samples used for this study were obtained at ages 19 years, 14 years, and 10 years, respectively. Serum was isolated and stored at  $-80^{\circ}\text{C}$ .

Patients diagnosed with ISS were recruited as part of a previously reported study examining the genetic basis of ISS [19]. Sera, isolated from blood samples, were stored in aliquots at  $-80^{\circ}\text{C}$ . The four male ISS subjects were of comparable ages to subjects P2 and P3.

### B. Plasma Transfusion Protocol

The plasma transfusion protocol was registered at ClinicalTrials.gov (no. NCT02412943). Fifteen samples of fresh-frozen plasma (FFP) were obtained from the local blood bank. PAPPA2 was measured by ELISA (Ansh Laboratories, Houston, TX). PAPPA2 was detectable in all the FFP samples with a mean value of  $0.28 \pm 0.06$  ng/mL. Based on these data, it was estimated that a 20 mL/kg FFP transfusion would lead to an increase in serum PAPPA2 levels of  $\sim 0.11$  ng/mL, assuming a plasma volume of 50 mL/kg and retention of all of the PAPPA2 in the plasma space. The analytical sensitivity of the PAPPA2 ELISA is 0.07 ng/mL.

P1 was given a total of 772 mL of FFP (19.3 mL/kg) from four separate units of FFP. PAPPA2 concentrations were measured from aliquots of each unit of FFP and ranged from 0.141 to 0.309 ng/mL. In total, the patient received  $\sim 170$  ng of PAPPA2. At the time of the transfusion, P1's hematocrit was 39%. Using an estimated total blood volume of 65 mL/kg for an adult female, her plasma volume was estimated to be 1586 mL [ $65 \text{ mL/kg} \times 40 \text{ kg} \times (1 - 0.39)$ ]. Assuming that all of the transfused PAPPA2 remained within the plasma space, we calculated that her plasma PAPPA2 concentration should have increased by 0.11 ng/mL. Using a similar calculation, the expected increases in total and free IGF-I concentrations due to the transfusion are 119 ng/mL and 3.5 ng/mL, respectively.

At the time of the transfusion protocol, P1 was 18 years old. All study visits were performed in the outpatient clinical research center at CCHMC. On day  $-1$ , a baseline sample was obtained for a complete blood count, blood type and cross, total IGF-I, total IGF-BP-3, PAPPA2, and free IGF-I. On day 0, repeat baseline measures were obtained and then the subject received a 20 mL/kg transfusion of FFP. She was observed in the clinic for 2 hours posttransfusion with no adverse events noted. She then returned to the outpatient clinic on days 1, 3, 7, 11, and 14 for repeat measurements of IGF-I, IGF-BP-3, PAPPA2, and free IGF-I. The total IGF-I and IGF-BP-3 levels for the transfusion protocol were performed in the CCHMC clinical laboratories using IDS-iSYS chemiluminescent immunoassays (Immuno-diagnostic Systems, Tyne & Wear, United Kingdom).

### C. rhPAPPA2 Expression and Purification

Wild-type PAPPA2 (Myc-DDK tagged)-human pappalysin 2 (PAPPA2), transcript variant 1 (NM\_020318) in pCMV6 (Origene, Rockville, MD) was maintained in DH5- $\alpha$  *Escherichia coli*

(Invitrogen, Carlsbad, CA). Plasmids were isolated with a plasmid maxi kit (Qiagen, Valencia, CA), and *PAPPA2* Sanger sequencing was performed to confirm the presence of the transcript. HEK293T cells were cultured in 10% fetal bovine serum/DMEM at 37°C in a humidified incubator with 5% CO<sub>2</sub>. At 60% to 70% confluence, HEK293 cells were transfected with pCMV6-PAPPA2 plasmid (35 µg per p150) using PolyJet transfection reagent (SigmaGen, Rockville, MD), following the manufacturer's protocol. After 24 hours, transfected cells were placed in serum-free media (0.1% BSA/DMEM) for 48 to 72 hours. Conditioned media were collected and combined with equal parts 1× PBS and passed over a 100-kDa cutoff Amicon Ultra-15 centrifugal filter (Millipore, Darmstadt, Germany) twice to purify PAPPA2. The purity of rhPAPPA2 was analyzed by immunoblots. Proteins in conditioned media were separated on 4% to 20% SDS gradient gels (Bio-Rad Laboratories, Hercules, CA), transferred to nitrocellulose membranes (GE Healthcare/Amersham Biosciences, Germany), and the membrane was first stained with a MemCode reversible protein stain kit (Pierce, Rockford, IL) to check general protein profile prior to immunoblotting with anti-PAPPA2 mouse monoclonal antibody (Abcam, Cambridge, MA) [20]. A human PAPPA2 ELISA was employed to measure the concentration of rhPAPPA2. Isolated rhPAPPA2 protein preparations were stored at –20°C.

#### D. In Vitro *rhPAPPA2* Enzyme Activities

The ability of the purified rhPAPPA2 to proteolyze IGFBP-3 and IGFBP-5 was evaluated. The source of the IGFBP-3 was media conditioned by human dermal fibroblasts, which we previously demonstrated secreted abundant IGFBP-3 [21]. rhIGFBP-5 was from GroPep (GroPep Bioreagents, Thebarton, Australia). rhPAPPA2 enzymatic activities were determined as follows: equal parts of fibroblast-conditioned media were incubated with non-concentrated rhPAPPA2 or vector control–conditioned media for 24 to 48 hours at 37°C. Two hundred nanograms of rhIGFBP5 was similarly processed. Cleavage of IGFBP-3 and rhIGFBP-5 was visualized by immunoblotting with anti-IGFBP-3 or anti-IGFBP-5 mouse monoclonal antibodies (Santa Cruz Biotechnology, Dallas, Texas, and GroPep, respectively) [22, 23]. rhPAPPA2 protease activity on endogenous IGFBP-3 in human serum was assessed by incubating 300 µL of patient or control serum with 0 ng, 5 ng, or 50 ng of rhPAPPA2 at 37°C for 0, 30, 60, and 240 minutes. The 0-ng group received an equal volume of diluent. The mixtures were snap frozen and stored at –20°C for ELISA analysis. Commercially available PAPPA2, free IGF-1, and intact IGFBP-3 ELISA kits (Ansh Laboratories) were employed to measure protein in the serum and rhPAPPA2 mixtures according to the manufacturer's protocols. Per the manufacturer, within-run and between-run percentage CV for PAPPA2 had a value of 4.25% and 1.94% for the low-serum control group (1.029 ng/mL) and 3.68% and 2.42% for the high-serum group (3.128 ng/mL). Intact IGFBP-3 had percentage CV for within run and between run for low control of 4.93% and 3.4% (22.069 ng/mL) and 2.88% and 3.05% for the high control (54.468 ng/mL). Additionally, free IGF-1 had a 4.14% within run and 4.81% between CV for low control (2.087 ng/mL) and 4.27% and 4.17% for high (8.191 ng/mL).

#### E. Statistical Analysis

Data were examined for distributional properties and detection of any outliers. Skewness was observed for both free IGF-I and intact IGFBP-3. Natural log and square root transformations were applied, respectively. No outlying values were detected and the 0 values were confirmed. Analysis was done on both raw and transformed values. Raw values are shown in all figures. A general linear mixed model was used to account for the repeated measures and a single missing data point. The initial approach was to examine a model for both of the outcome variables (bioavailable IGF-1 and intact IGFBP-3) and include time (0, 30, 60, and 240 minutes), dose (0 ng, 5 ng, and 50 ng) and group (ISS and PAPPA2 deficiency) in the model and examine the interaction. Time was considered as continuous in the model. In the event of significant interactions between time, dose and group, or time and dose we planned to

consider separate models by dose. Analysis was done using SAS<sup>®</sup>, version 9.4 (SAS Institute, Cary, NC). A *P* value of <0.05 was considered statistically significant. Due to the nature of the study being to provide proof-of-principle evidence, no adjustment was made for multiple comparisons. Of note, the same results were obtained using both raw and transformed variables, so results from the analysis of the raw data are presented.

## 2. Results

### A. Plasma Transfusion for *in Vivo* PAPP A2 Replacement

Prior to transfusion, P1 had a mean PAPP A2 concentration of 0.275 ng/mL. We have previously shown that this subject's mutated PAPP A2 is not proteolytically active [14]. At day 1 posttransfusion, there was no significant measurable increase in PAPP A2 concentration (Fig. 1). However, despite the lack of measurable increase in PAPP A2, free IGF-I levels increased 2.5-fold, with a gradual return to baseline during the 14-day period (Fig. 1). A *t* test showed a statistically significant mean change between the baseline values (days -1 and 0) and postbaseline values (days 1 to 14) (*P* = 0.04). Examining the 95% CI for the postbaseline values showed that the baseline values fall outside the CI (mean, 3.35; 95% CI, 2.40, 4.30). There was no significant change in total IGF-I, total IGFBP-3, or intact IGFBP-3 throughout the study period (Fig. 1; Supplemental Table 1).

### B. *In Vitro* rhPAPP A2 Functional Validation

To further explore the effects of PAPP A2 on free IGF-I levels *in vitro*, we generated rhPAPP A2 peptides by overexpressing pCMV6-PAPP A2 in HEK293 cells. Secreted rhPAPP A2 was readily immunodetected in the conditioned media (Fig. 2A, lane 2) and was more prominent after concentration (Fig. 2A, lane 3). Retention of enzyme activity was confirmed by loss of detectable intact IGFBP-3 and rhIGFBP-5 when incubated with rhPAPP A2 (Fig. 2B and 2C), consistent with our previous report [14].

### C. rhPAPP A2 Proteolyzes IGFBPs in Serum Samples

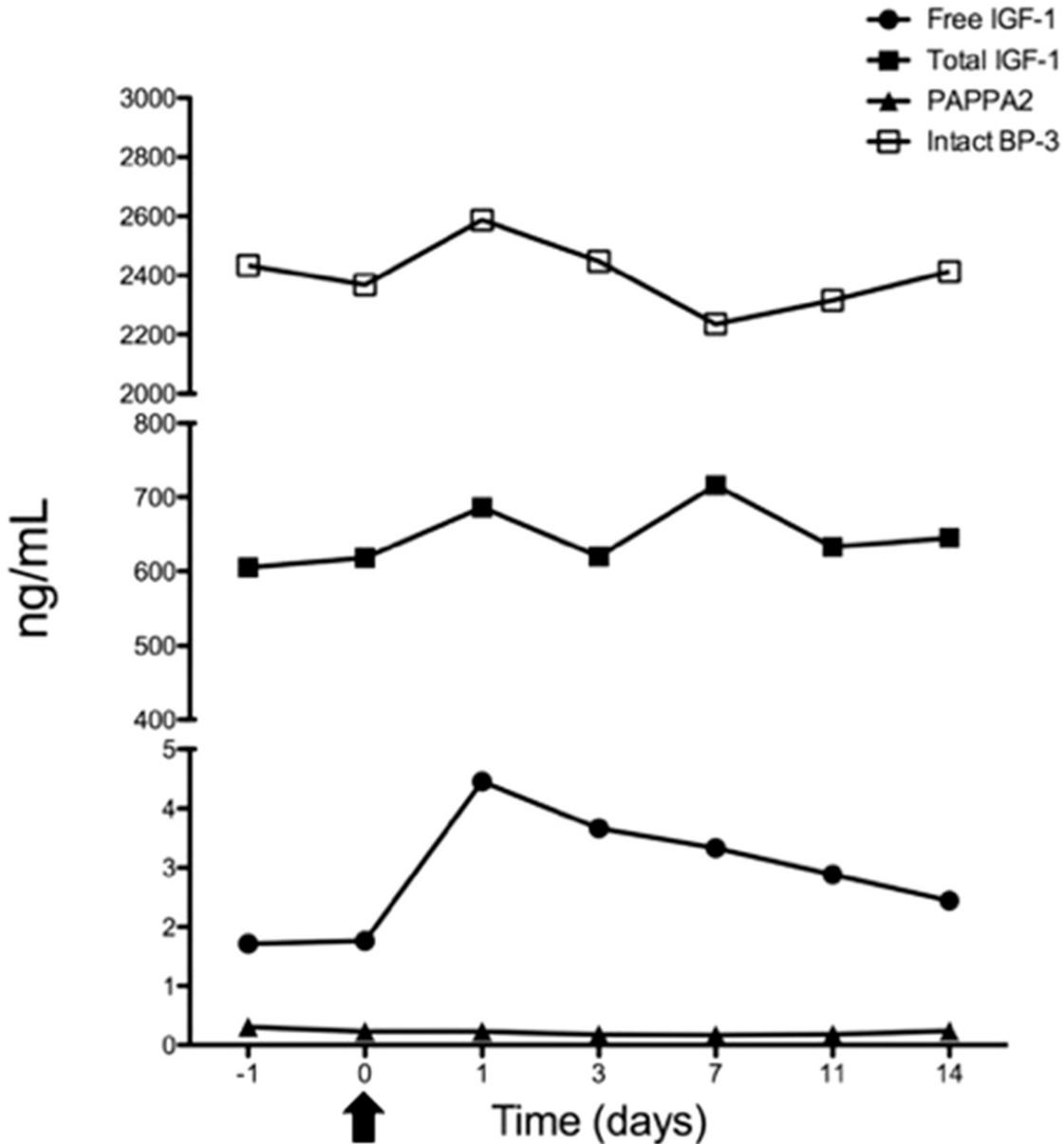
To validate our hypothesis that rhPAPP A2 will increase free IGF-I levels in patients with PAPP A2 deficiency who have low free IGF-I and high levels of total IGF-I, we combined purified rhPAPP A2 with serum samples obtained from the three affected siblings (P1, P2, and P3). We also explored whether rhPAPP A2 could have similar effects in serum samples from patients with ISS who are not PAPP A2 deficient.

In serum samples from our PAPP A2-deficient patients, intact IGFBP-3 ( $2604 \pm 69$  ng/mL to  $2666 \pm 76$  ng/mL; *P* = 0.42) and free IGF-I ( $1.85 \pm 1.37$  ng/mL to  $2.36 \pm 1.77$  ng/mL; *P* = 0.57) levels remained stable during the course of 4 hours without the addition of rhPAPP A2 (Fig. 3). Addition of 5 or 50 ng of rhPAPP A2 to patient serum samples markedly and dose-dependently decreased intact IGFBP-3 levels compared with the baseline time point (Fig. 3). Concurrently, increased free IGF-I concentrations between 15- and 100-fold were detected (*P* = 0.001 for 5 ng and *P* = 0.008 for 50 ng) (Fig. 3; Table 1).

Similar trends were seen for patients with ISS, although the degree of increase in free IGF-I was more modest (1.7- to 5-fold) (Fig. 3). Note that the patients with ISS had lower starting total IGF-I, total IGFBP-3 and intact IGFBP-3, but higher free IGF-I levels, when compared with PAPP A2-deficient patients (Table 1). Hence, the baseline free-to-total IGF-I ratios were markedly higher in the ISS patients. Statistical analysis supported the significantly greater increase in free IGF-I for the PAPP A2 group compared with the ISS group (*P* = 0.0005 for 5 ng and *P* = 0.01 for 50 ng).

## 3. Discussion

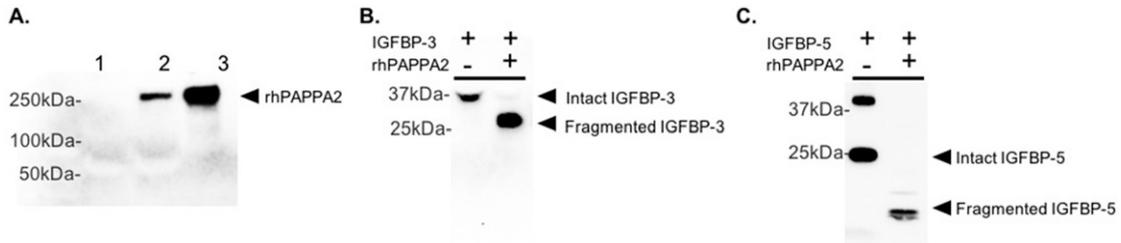
To our knowledge, this is the first study to explore the potential use of PAPP A2 as a modulator of IGF-I bioavailability in patient samples. PAPP A2 has been shown to be a key



**Figure 1.** Biochemical measurements during plasma transfusion experiment. Serum was collected 1 day prior to infusion (–1), at baseline on the day of transfusion (0), and on days 1, 3, 7, 11, and 14 after transfusion. Arrow indicates the transfusion on day 0.

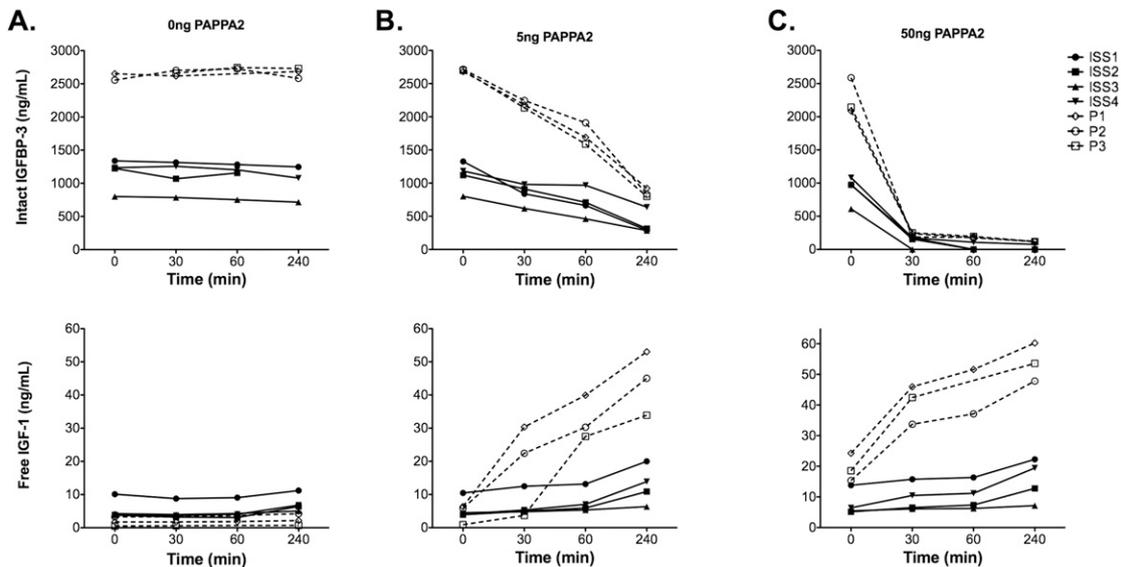
regulator of IGF-I bioavailability, and the recently described patients with loss-of-function *PAPPA2* mutations demonstrated postnatal growth retardation with low free IGF-I levels [14]. We hypothesized that administration of exogenous *PAPPA2* would proteolyze IGF-BP-3 molecules, thereby freeing up endogenous IGF-I from its ternary complex. Theoretically, higher levels of free IGF-I could lead to increased growth over time.

We used two complementary approaches to investigate the effect of augmenting *PAPPA2* activity in patients with *PAPPA2* deficiency, extending our study to include samples from patients with ISS. In the first approach, we delivered a relatively small dose of *PAPPA2* via plasma transfusion to a single patient with *PAPPA2* deficiency. Unfortunately, there was no detectable increase in the serum *PAPPA2* concentration posttransfusion. There are a number of possible explanations for this lack of increase. It is possible that there was a small increase in *PAPPA2* levels that fell below the discriminatory threshold of the assay. Our maximal



**Figure 2.** A. Immunoblot analyses of rhPAPPA2 expression and proteolytic activities. (A) Conditioned media (CM) from vector-transfected (lane 1) or pCMV6-PAPPA2-transfected (lane 2) HEK293 cells. Lane 3, rhPAPPA2 after concentration. (B) Secreted intact IGFBP-3 in media conditioned by human dermal fibroblasts was incubated with or without rhPAPPA2 (~20 ng) for 24 h at 37°C. Addition of rhPAPPA2 resulted in cleavage of IGFBP-3. (C) rhPAPPA2 was incubated for 48 h at 37°C with rhIGFBP5. Immunoblot showing cleavage of IGFBP-5 in the presence of rhPAPPA2.

expected increase was 0.11 ng/mL and the analytical sensitivity of the assay is only 0.07 ng/mL. It is also possible that the PAPPA2 was metabolized before the day 1 measurement or was distributed into other tissues and thus the expected increase in plasma levels was not observed. Despite this lack of measurable increase in PAPPA2, the free IGF-I levels did increase 2.5-fold, with a slow return to baseline. This suggests that even a small transient increase in PAPPA2 levels may be sufficient for a prolonged effect on free IGF-I levels. The transfusion did also include some free IGF-I, but we think the persistent increase in free IGF-I is unlikely to be due to transfused free IGF-I, as the half-life of free IGF-I *in vivo* is only a few minutes. Interestingly, intact IGFBP-3 levels did not fall during the course of the experiment, which questions whether the increase in free IGF-I levels was a consequence of PAPPA2 actions on IGFBP3. PAPPA2 is known to efficiently proteolyze IGFBP-5, which was not measured during this trial. Additional *in vivo* studies are needed to determine whether PAPPA2 replacement could have a sustained effect on increasing free IGF-I levels.



**Figure 3.** Intact IGFBP-3 and free IGF-1 values in serum from PAPPA2-deficient patients (P1, P2, and P3) and ISS patients (ISS1, ISS2, ISS3, and ISS4) after 0, 30, 60, and 240 min of incubation at 37°C with rhPAPPA2. The 0 min time point occurred after incubation periods for each assay, thus resulting in different baseline free IGF-1 and intact IGFBP-3 values for the same samples at the different doses of rhPAPPA2 (*i.e.*, there was some enzyme action during the incubation). (A) Addition of 0 ng of rhPAPPA2. (B) Addition of 5 ng of rhPAPPA2. (C) Addition of 50 ng of rhPAPPA2.

**Table 1. Demographic and Baseline Hormonal Parameters for Patients Carrying the PAPP A2 Mutation and Patients Diagnosed with ISS**

	P1	P2	P3	ISS1	ISS2	ISS3	ISS4
Sex	F	M	M	M	M	M	M
Age, y	19.3	14.5	10.5	14	10.5	13.5	8.5
Height, SD	-3.8	-2.9	-2.9	-2.5	-2.1	-2.4	-2.5
Tanner stage	5	2	1	1	1	2	1
Total IGF-I, ng/mL	566	573	547	299	169	102	137
Total IGFBP-3, ng/mL	7924	8997	>10,000	4111	4319	3099	3658
Free IGF-I, ng/mL	1.75	3.27	0.53	10.11	3.78	4.28	3.85
Intact IGFBP-3, ng/mL	2653	2556	2493	1338	1224	801	1235
Free IGF-I/IGF-I ratio, %	0.3	0.6	0.1	3.3	2.2	4.2	2.8
PAPP A2, ng/mL	0.09	0.06	0.11	0.51	0.19	0.16	0.29

Tanner Stage was based on testicular volume for males and breast for females.

Our second approach involved *in vitro* studies, exploring the effect of rhPAPP A2 on intact IGFBP-3 and free IGF-I levels in serum from patients with either PAPP A2 deficiency or ISS. These results showed that PAPP A2 administration leads to rapid cleavage of IGFBP-3 and significant rises in free IGF-I concentrations, supporting our initial hypothesis. The increase in free IGF-I was significantly more prominent in patients with PAPP A2 deficiency as expected, as these patients have greater stores of bound IGF-I poised to be liberated from their ternary complexes. The ISS patients also demonstrated significant increases in their free IGF-I levels, albeit more modest, ranging from 1.7- to 5-fold. Interestingly, the serum sample from the one ISS patient with the highest free IGF-I to total IGF-I ratio at baseline has the smallest increase in free IGF-I upon the addition of PAPP A2. Presumably, this patient had a smaller fraction of bound IGF-I available for rhPAPP A2 actions. Although our analyses are all *in vitro* and involved a limited number of patient serum samples, the results do raise the intriguing possibility that PAPP A2 therapy could lead to a meaningful increase in free IGF-I levels in patients with short stature. Currently, there is a lack of information about the pharmacokinetics or pharmacodynamics of PAPP A2, and thus much additional work is necessary before PAPP A2 can be considered as a viable therapeutic option for short stature. Although the normal ranges for PAPP A2 or free IGF-I in childhood need to be established to fully interpret the clinical impact of our results, our data support the potential of the development of PAPP A2 as a novel growth-promoting therapy.

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**Disclosure Summary:** A.D. and V.H. hold a patent on the use of PAPP A2 as a growth-promoting agent. The remaining authors have nothing to disclose.

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