

## Mutations in pregnancy-associated plasma protein A2 cause short stature due to low IGF-I availability

Andrew Dauber, María T. Muñoz-Calvo, Vicente Barrios, Horacio M. Domené, Soren Kloverpris, Clara Serra-Juhé, Vardhini Desikan, Jesús Pozo, Radhika Muzumdar, Gabriel Á. Martos-Moreno, Federico Hawkins, Héctor G. Jasper, Cheryl A. Conover, Jan Frystyk, Shoshana Yakar, Vivian Hwa, Julie A. Chowen, Claus Oxvig, Ron G. Rosenfeld, Luis A. Pérez-Jurado, Jesús Argente

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	Accepted:	25 January 2016

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### Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

*Editor: Céline Carret*

1st Editorial Decision

11 January 2016

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine and please accept my sincere apologies for the delay in getting back to you, due to the festive season. We have now received the enclosed reports from the referees who assessed your article. As you will see, the reviewers are globally supportive and I am pleased to inform you that we will be able to accept your manuscript pending the following final amendments:

1) please address all minor issues highlighted by the reviewers and provide a point-by-point response to their comments (as word file).

Please submit your revised manuscript as soon as possible.

\*\*\*\*\* Reviewer's comments \*\*\*\*\*

Referee #1 (Comments on Novelty/Model System):

The finding of a new syndrome due to PAPP2 mutations is an important contribution to pediatric endocrinology and growth homeostasis. It underscores the physiological importance of a new effector of IGF1 transport and raises novel questions about growth regulatory pathways. Genetics

are strongly supported by clear functional demonstration of biochemical defects.

Referee #1 (Remarks):

The finding of a new syndrome due to PAPP2 mutations by Dauber et al is an important contribution to the physiology of growth and the mechanisms that can alter IGF1 transport and effects on growth plates. Genetic findings are clear. functional experiments that show the biochemical consequences of the mutations in vitro and in vivo provide a strong and undisputable support to the genotype phenotype relationship. The paper is well written and concise.

I would only suggest micro modifications.

Page 8: replace "extremely elevated" by a more appropriate wording

Page 10: "This is a unique....in human short stature" is not true: children with PROP1 mutations revealing late in childhood, as well as children with mild forms of SHOX mutations may well show this kind of growth trajectories. I would simply skip this sentence.

Discussion: The authors should give their opinion and possibly their experience about treatment issues, notably the use of recombinant IGF1.

Referee #2 (Comments on Novelty/Model System):

This is generally a well-written manuscript describing a novel molecular mechanism that accounts for an unusual short stature phenotype in 2 distinct human pedigrees. Biochemical data have been reproduced satisfactorily in a pre-existing mouse model. Overall this manuscript has significantly advanced knowledge in the field and is worthy of publication.

Referee #2 (Remarks):

This is an interesting and well-written manuscript that details the documentation of homozygous mutations in PAPP-A2 in 2 pedigrees with short stature. The authors have provided good genetic evidence as well as functional analysis of the mutations that implicate the gene in the aetiology of the short stature. The mechanism is also plausible - mutations in PAPP-A2 interfere with cleavage of IGFBP3 and IGFBP5 and hence although the total IGF1 concentrations are high, the concentration of fIGF1 is low. This would then lead to impaired growth. I would suggest that the following changes be considered:

1. Introduction: I would suggest that the authors replace the word "correct human growth" by "optimal human growth".
2. The authors need to state that HEK293 cells were used in the Methods.
3. In the Results section, at what time was the secretion of GH assessed - was it during the night?
4. The growth velocity in Figure 1A and 1B does not appear to be very slow, whereas in Pedigree 2, the growth velocity appears to be much slower. Additionally, in Figure 1D, the growth rate is slow with an increased weight gain. This could be associated with the hyperinsulinaemia - were serial measurements made of insulin concentrations in patient II.4 in Pedigree 2?
5. Suggest that "concentrations" should be used instead of "levels", and decimal ages should be used throughout.
6. It would be important to document the fact that the SGA is relative - and pedigree specific. Affected children had lower birth weights than unaffecteds.
7. The manuscript would be enhanced by the addition of DXA and skeletal survey data in Pedigree 2. Were there any tooth abnormalities in Pedigree 2.
8. Could the authors discuss a potential role for recombinant human IGF1 therapy?
9. It would appear that the growth failure appears to be mainly pubertal - could the authors comment on this phenomenon?

We would like to thank both referees for their positive comments and constructive suggestions that helped us to improve the quality of this manuscript.

*Referee #1 (Remarks):*

*The finding of a new syndrome due to PAPP2 mutations by Dauber et al is an important contribution to the physiology of growth and the mechanisms that can alter IGF1 transport and effects on growth plates. Genetic findings are clear. functional experiments that show the biochemical consequences of the mutations in vitro and in vivo provide a strong and undisputable support to the genotype phenotype relationship. The paper is well written and concise.*

*I would only suggest micro modifications.*

*Page 8: replace "extremely elevated" by a more appropriate wording*

Response: We have modified this statement as suggested.

*Page 10: "This is a unique...in human short stature" is not true: children with PROP1 mutations revealing late in childhood, as well as children with mild forms of SHOX mutations may well show this kind of growth trajectories. I would simply skip this sentence.*

Response: We have removed this statement as suggested.

*Discussion: The authors should give their opinion and possibly their experience about treatment issues, notably the use of recombinant IGF1.*

Response: We have included a statement regarding the use of recombinant IGF-I in these patients (Page 11).

*Referee #2 (Comments on Novelty/Model System):*

*This is generally a well-written manuscript describing a novel molecular mechanism that accounts for an unusual short stature phenotype in 2 distinct human pedigrees. Biochemical data have been reproduced satisfactorily in a pre-existing mouse model. Overall this manuscript has significantly advanced knowledge in the field and is worthy of publication.*

*Referee #2 (Remarks):*

*This is an interesting and well-written manuscript that details the documentation of homozygous mutations in PAPP-A2 in 2 pedigrees with short stature. The authors have provided good genetic evidence as well as functional analysis of the mutations that implicate the gene in the aetiology of the short stature. The mechanism is also plausible - mutations in PAPP-A2 interfere with cleavage of IGFBP3 and IGFBP5 and hence although the total IGF1 concentrations are high, the concentration of fIGF1 is low. This would then lead to impaired growth. I would suggest that the following changes be considered:*

*1. Introduction: I would suggest that the authors replace the word "correct human growth" by "optimal human growth".*

Response: This has been changed as suggested.

*2. The authors need to state that HEK293 cells were used in the Methods.*

Response: This information has been included in the Materials and Methods (Page 15).

*3. In the Results section, at what time was the secretion of GH assessed - was it during the night?*

Response: This analysis was performed during the day. This is now included in the methods section.

*4. The growth velocity in Figure 1A and 1B does not appear to be very slow, whereas in Pedigree 2, the growth velocity appears to be much slower. Additionally, in Figure 1D, the growth rate is slow with an increased weight gain. This could be associated with the hyperinsulinaemia - were serial measurements made of insulin concentrations in patient II.4 in Pedigree 2?*

Response: There is a clear deterioration of the growth velocity in both patients of Family 1 in relationship to their target height (P50-75). Regarding the possibility of hyperinsulinaemia in patient II.4 in Family 2, this is of course a good observation. We also believe that this is a possibility. However, serial measurements of insulin concentrations have not been performed in this patient.

*5. Suggest that "concentrations" should be used instead of "levels", and decimal ages should be used throughout.*

Response: We have made the suggested changes.

*6. It would be important to document the fact that the SGA is relative - and pedigree specific. Affected children had lower birth weights than unaffected.*

Response: We have clarified this in the manuscript. None of the patients were classified as SGA, according to the International Consensus. The male patient in pedigree 1 was born premature, but adequate for gestational age, followed by normal catch-up growth. This can be appreciated in Annex Figure 1.

*7. The manuscript would be enhanced by the addition of DXA and skeletal survey data in Pedigree 2. Were there any tooth abnormalities in Pedigree 2.*

Response: Unfortunately we do not have DXA data in Pedigree 2 at the moment of diagnosis. In contrast, the skeletal survey data was performed and some of this data are represented in Figure 1.

We did not have the opportunity to study any tooth in this family as of yet.

*8. Could the authors discuss a potential role for recombinant human IGF1 therapy?*

Response: This has been included in the Discussion section (Page 11). Patients in Family 1 are currently under human recombinant IGFI therapy.

*9. It would appear that the growth failure appears to be mainly pubertal - could the authors comment on this phenomenon?*

Response: The auxological data indicate that there is a slow deterioration of growth velocity prepubertally. Indeed, it appears that this deterioration of growth velocity is accentuated pubertally. However, as all patients have not yet reached puberty, it is difficult to make a statement in this regard. Hence, we would prefer not to make this generalization until all patients have reached puberty.

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

Corresponding Author Name: Jesús Argente  
 Journal Submitted to: EMBO Molecular Medicine  
 Manuscript Number: EMM-2015-06106

### Reporting Checklist For Life Sciences Articles (Rev. July 2015)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

#### A- Figures

##### 1. Data

###### The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if  $n < 5$ , the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

##### 2. Captions

###### Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple  $\chi^2$  tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of "center values" as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

#### B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	The test sample size was determined by the number of individuals that presented mutations in the gene under study and their siblings. Formal statistical testing for power calculation is not applicable to this study.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	N/A
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	N/A
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	N/A
For animal studies, include a statement about randomization even if no randomization was used.	N/A
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	N/A
4.b. For animal studies, include a statement about blinding even if no blinding was done	N/A
5. For every figure, are statistical tests justified as appropriate?	N/A
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	N/A
Is there an estimate of variation within each group of data?	N/A
Is the variance similar between the groups that are being statistically compared?	N/A

#### C- Reagents

#### USEFUL LINKS FOR COMPLETING THIS FORM

<http://www.antibodypedia.com>  
<http://1degreelibio.org>  
<http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo>  
  
<http://grants.nih.gov/grants/olaw/olaw.htm>  
<http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm>  
<http://ClinicalTrials.gov>  
<http://www.consort-statement.org>  
<http://www.consort-statement.org/checklists/view/32-consort/66-title>  
  
<http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun>  
  
<http://datadryad.org>  
  
<http://figshare.com>  
  
<http://www.ncbi.nlm.nih.gov/gap>  
  
<http://www.ebi.ac.uk/ega>  
  
<http://biomodels.net/>  
  
<http://biomodels.net/miriam/>  
<http://iji.biochem.sun.ac.za>  
[http://oba.od.nih.gov/biosecurity/biosecurity\\_documents.html](http://oba.od.nih.gov/biosecurity/biosecurity_documents.html)  
<http://www.selectagents.gov/>

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia ( <a href="#">see link list at top right</a> ), 1DegreeBio ( <a href="#">see link list at top right</a> ).	page 16, reference Winn et al.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	page 15

\* for all hyperlinks, please see the table at the top right of the document

#### D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	page 17
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	page 17
10. We recommend consulting the ARRIVE guidelines ( <a href="#">see link list at top right</a> ) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH ( <a href="#">see link list at top right</a> ) and MRC ( <a href="#">see link list at top right</a> ) recommendations. Please confirm compliance.	page 17

#### E- Human Subjects

11. Identify the committee(s) approving the study protocol.	The Institutional Review Boards at the Hospital Infantil Universitario Nino Jesus and Cincinnati Children's Hospital Medical Center approved these studies. Page 11.
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	This has been included in the manuscript. Page 11
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	Consent to publish photos was obtained. This is included on Page 11.
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	There are no restrictions on use of the human data or samples.
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram ( <a href="#">see link list at top right</a> ) and submit the CONSORT checklist ( <a href="#">see link list at top right</a> ) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	N/A
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines ( <a href="#">see link list at top right</a> ). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	N/A

#### F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition'.  Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	N/A
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad ( <a href="#">see link list at top right</a> ) or Figshare ( <a href="#">see link list at top right</a> )).	N/A
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP ( <a href="#">see link list at top right</a> ) or EGA ( <a href="#">see link list at top right</a> )).	Exome sequencing was performed in one individual in each of the two families as described in the manuscript. We are publishing full facial photos with their permission, but that makes these patients identities easily recognizable. Therefore, we would prefer not to post their individual exome data to a public repository.
21. As far as possible, primary and referenced data should be formally cited in a Data Availability section. Please state whether you have included this section.  Examples: <b>Primary Data</b> Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in <i>Shewanella oneidensis</i> MR-1. Gene Expression Omnibus GSE39462 <b>Referenced Data</b> Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	We have not included this section as it is not applicable to our study.
22. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines ( <a href="#">see link list at top right</a> ) and deposit their model in a public database such as Biocompare ( <a href="#">see link list at top right</a> ) or JWS Online ( <a href="#">see link list at top right</a> ). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N/A

#### G- Dual use research of concern

23. Could your study fall under dual use research restrictions? Please check biosecurity documents ( <a href="#">see link list at top right</a> ) and list of select agents and toxins (APHIS/CDC) ( <a href="#">see link list at top right</a> ). According to our biosecurity guidelines, provide a statement only if it could.	N/A
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