EMBO Molecular Medicine

A spoonful of L-fucose - An efficient therapy for GFUS-CDG, a new glycosylation disorder

René Feichtinger, Andreas Hüllen, Andreas Koller, Dieter Kotzot, Valerian Grote, Erdmann Rapp, Peter Hofbauer, Karin Brugger, Christian Thiel, Johannes Mayr, and Saskia Wortmann
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(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. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

1st Editorial Decision 11th May 2021

11th May 2021

Dear Dr. Wortmann,

Thank you again for submitting your work to EMBO Molecular Medicine. We have now heard back from the four referees who evaluated your manuscript. As you will see from the reports below, the referees acknowledge the potential interest of the study. However, they also raise a series of concerns about your work, which should be convincingly addressed in a major revision of the present manuscript.

The referees' recommendations are rather clear and there is no need to reiterate their comments. All issues raised by the referees would need to be convincingly addressed. In particular, during our pre-decision cross-commenting process (in which the referees are given a chance to make additional comments, including on each other's reports), Referees #1 and #2 agreed with Referee #4's suggestion to increase the number of control samples.

We would welcome the submission of a revised version within three months for further consideration. Please note that EMBO Molecular Medicine strongly supports a single round of revision. As acceptance or rejection of the manuscript will depend on another round of review, your responses should be as complete as possible.

EMBO Molecular Medicine has a "scooping protection" policy, whereby similar findings that are published by others during review or revision are not a criterion for rejection. Should you decide to submit a revised version, I do ask that you get in touch after three months if you have not completed it, to update us on the status.

We are aware that many laboratories cannot function at full efficiency during the current COVID-19/SARS-CoV-2 pandemic and have therefore extended our "scooping protection policy" to cover the period required for a full revision to address the experimental issues. Please let me know should you need additional time, and also if you see a paper with related content published elsewhere.

Please read below for important editorial formatting and consult our author's guidelines for proper formatting of your revised article for EMBO Molecular Medicine.

I look forward to receiving your revised manuscript.
Jingyi

Jingyi Hou
Editor
EMBO Molecular Medicine

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

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

The study described a novel CDG caused by GDP-L-fucose deficiency (GFUS mutation) in a child with developmental disability, growth retardation and dysmorphic facial features. GFUS protein and GDP-L-fucose were reduced leading to glycoprotein hypofucosylation. Moreover, the study shows that the conversion of GDP-D-[2- 3H]mannose to GDP-L-[2- 3H]fucose was dramatically reduced in patient-derived fibroblasts. Fucosylation was restored using retroviral complementation with the wildtype GFUS-cDNA of patient-derived fibroblasts which ascertained the causative effects of the variants detected.
The study well describes the effect of L-fucose supplementation on protein fucosylation in vitro and in vivo.

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The study well describes the effect of L-fucose supplementation on protein fucosylation in vitro and in vivo.
Fucose supplementation restored fucosylation in patient-derived fibroblasts stained with AAL lectin.
The study interestingly shows that oral L-fucose supplementation was followed by improved fucosylation of proteins and some clinical improvements. Overall the study harbors a considerable novelty in the field and deserves particular interest in the light of the explored therapeutic approach.

Revisions required

1. The introduction briefly reports some essential elements of glycobiology with particular regard to the biosynthesis of GDP-L-fucose which improves comprehension to the non-specialist readers. However, information on CDG first-line screening test (i.e. serum transferrin IEF) and related pitfalls is particularly envisaged at this point.
Actually, this novel CDG adds to the list of CDG with normal serum transferrin IEF.

2. When citing therapeutic strategies in known CDG, it would be interesting to briefly report the different circumstances that make useful some monosaccharides as potential treatments in CDG.

Patient description
- I understand that reported prenatal information are related to fetal (child?) growth and fetal movements.

- Definition of "cerebral cysts" detected prenatally is vague and not sufficiently documented (i.e. localization, evolution during pregnancy are some information that should be added). -- Additional information on post-natal brain appearance as detected by newborn cerebral ultrasound might be meaningful also in the light of the increased head circumference at birth (97th pc).
- As the child was affected with a neurodevelopmental disorder, some information on possible seizure disorder and patient electroclinical features should be reported in the patient clinical description.

Biochemical and molecular investigations
- the mRNA expression levels for the Golgi GDPfucose transporter SLC35C1 (54.4% {plus minus} 6.5%; p=***0.00003) as well as for the potential ER GDP-fucose transporter SLC35C2 (63.3% {plus minus} 17.4%; p=*0.035 (Fig.4B)) were significantly diminished. Is there any explanation for this finding? This is particularly important to address the use of sugar supplementation as well.

- The effect of L-fucose supplementation on O-fucosylation is not adequately described in the results section. Please underline the strategies used to detect baseline deficiency and post-fucose modification of O-fucosylated proteins.

Clinical course after 19 months of oral L-fucose supplementation
- The clinical course was monitored by growth parameters and standardized psychological tests. It would be beneficial to know if CDG severity scoring was evaluated before and during treatment (i.e. Nijmegen CDG rating scale).

- Although there was a considerable improvement in mental capacities on psychological tests, it would be interesting to add some information on modification of age-related activities of daily living, if any.

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

Regarding the TECHNICAL QUALITY, my major concerns are about the xCGE-LIF analysis of N-glycome and FACS analysis of fucosylation of PBMCs.
- For these two points, although the presented data are finally rather convincing, corresponding (supplemental) Figure legends are overall unclear and very difficult to understand.

More precisely,
For xCGE-LIF,
- the number of controls (n=2) is unsatisfactory.
- thus, there is no corresponding statistical evaluation of results.
- nomenclature of N-glycans (A? G? D?...) should be clarified
- the claimed shift in the relative composition of the N-glycome is unclear viewing the 'difficult' Table EV1 (and not 'supplementary table 2' as mentioned?)
- why the announced increase of hybrid-type N-glycans not linked to detectable transferrin glycosylation defects?

For FACS analysis of the fucosylation of PBMCs,
- references to figure 5 and to suppl Fig3 (3 pages) seem wrong and are confusing. For example, fig 5C (8 weeks fucose) is mentioned for 2 weeks fucose in the Fig legend?

- Legend of Fig EV3 is very unclear

In addition,
FigEV4 is never mentioned in the manuscript? Is there a missing page in the submitted text?

Regarding the Fig. 5A (AAL lectin blot), I'm surprised that only 3 protein bands (what MW?) seem fucosylated in serum... Furthermore, legends of fig 5B/5C should be clarified.

NOVELTY is high since Feichtinger and col. described here a new treatable CDG.

MEDICAL IMPACT could be rather high (medium?) for this probably treatable CDG that concerns very few affected individuals. But evaluated clinical test(s) are rather scarce with rather slight improvements...

MODEL SYSTEM (fibroblasts, serum, platelets, PBMCs and the affected girl) are adequate.

Referee #2 (Remarks for Author):

Review of the manuscript entitled: A spoonnful of L-fucose - An efficient therapy for GFUS-CDG, a new glycosylation disorder, by Feichtinger and col.

This paper describing a new potentially treatable CDG is interesting (high novelty and potential medical impact for affected individuals) and well-documented (technical issues; literature)but needs to be strongly clarified and corrected.
In addition, I think that a more concise version should be proposed as a short report in EMBO Molecular Medicine.

MAJOR REMARKS:
My major concerns are about the xCGE-LIF analysis of N-glycome and FACS analysis of fucosylation of PBMCs.
- For these two points, although most of the presented data (xCGE-LIF?) are finally rather convincing, corresponding (supplemental) figure legends are overall unclear and very difficult to follow and understand.

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Concerning the text of the manuscript (essentially Discussion):
- I feel that the reader could be very confused between O-fucosylation and fucosylation of O-glycans (e.g. GalNAc O-glycans).
- Bombay group should be further explained. Unclear point.
- 'expression of SLC35C1 and FUT8 was found to be reduced in QRT-PCR studies' : where are the related data for FUT8? I did not find.
- Why the accumulation of FA3G3S1(2,6) (??) is interesting?
- 'expression of POFUT1 and POFUT2 were normal in qRT-PCR studies': where are the related data? I did not find.
- what about the described elevated protein level of FUT8?

MINOR POINTS:
- 'Kaufmann ABC' is unclear in the abstract.
- GAG (O-xyl) should be mentioned in the introduction.
- 'From bitrh on lack of appetite' (case reports): I don't understand.
- what were the tested prediction programs for gene variant pathogenicity?
- how long for 100 µM L-fucose treatment? (results).
- ADAMTS13 should be clarified in the results part: ADAMTS13, a heavily O-fucosylated protein...
- 'This could be due to the functional redundancy of the salvage and the de novo pathway': unclear.

Referee #3 (Remarks for Author):

This is a well-written manuscript on a novel CDG.
I have no problem with the content. The following are a few small corrections:
ABSTRACT
L 4: delay, mild
L 5: synthase,
L 8: serum glycoproteins, leukocytes
L 12: serum glycoproteins
INTRODUCTION
L 1: Some 2 %
L 6: anchored protein synthesis
L 11, 12: guanosine (not guanidine!)
L 23: CDG (is singular as well as plural)
10th last sentence: CDG no therapeutic
RESULTS
P 2, L 17: GDP-D-mannose
eight last line: no side effects

Referee #4 (Remarks for Author):

The manuscript details the identification of compound heterozygous missense mutations in GFUS encoding GDP-L-fucose synthase. This led to a novel CDG. The authors also show that fucose supplementation was at least partially effective as a treatment. The manuscript is well written and concise. The two aspects of the work, namely i) the identification of a new CDG, GFUS-CDG, and ii) the clinical study on the effectiveness of treatment with fucose are valuable to the scientific and clinical community.

With regards to i) the authors present a convincing case reinforced by reduced GFUS protein levels and a greatly decreased activity of GFUS in patient fibroblasts, as measured by the conversion of GDP-D-[2-3H]mannose to GDP-L-[2-3H]fucose. In combination clinical data that shows a broadly similar picture to existing disorders of fucose metabolism, this leads the reviewer to conclude that the GFUS variants in the patient presented are indeed causative for their disorder.
With regards to ii) the data is also relatively convincing due to lectin staining and fucosylation results from patient samples, as well as limited improvement in the clinical course. So far, only one patient is described. The authors address briefly why there haven't been any other patients identified, because the GFUS-/- mouse is nonviable and in this patient there is 1% residual activity, allowing some small amount of de novo fucose synthesis. More severe mutations would thus be lethal.

Major comments:

• For several data panels and results it seems only one control was used to compare with that of the patient (e.g. EV Fig 2.) Ideally two or several controls would be used for comparison, particularly for an inherently variable method such as western blotting, and bearing in mind only one patient was available so statistical analysis was already very difficult. If the authors in fact used several controls and the data shown was purely for presentation reasons, it should be stated clearly in the text. Particularly with regards to EV Fig 2, this makes it very hard to draw conclusions from the comparison of a single control with a single patient. This should at the very least be noted.

Minor comments:

• Abstract/introduction: The type of variant identified in the patients is not specified beyond 'biallelic' before the results section. The authors should add that the variants are compound heterozygous missense mutations to the abstract and/or introduction.
• In the discussion it is mentioned that when measured by qPCR, lower levels of transcripts from FUT8 were found. Firstly, this is not mentioned in results and should be added. Secondly, does this not oppose the higher FUT8 protein levels found by western blot? Can you explain this discrepancy?
• The authors also present qPCR data showing that GFUS, SLC35C1, SLC35C2 were all downregulated on a transcriptional level and describe this as a possible compensatory mechanism. Is there evidence of this in other defects of the fucose metabolic pathway?
• Do the authors think that in this case, with some residual enzymatic activity of GFUS, there is potential for supplementation with mannose (or analogue) as a substrate therapy? Or would this be unlikely to improve on the results already achieved by fucose therapy?
• Park et al. (https://pubmed.ncbi.nlm.nih.gov/33312876/) recently reported that fucose treatment was partially successful in a case of FUT8-CDG. Although their study was only on two patients and showed very tentative results, it is very relevant to this manuscript and should be at least

mentioned.

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

REFEREE #1 (COMMENTS ON NOVELTY/MODEL SYSTEM FOR AUTHOR):

The study described a novel CDG caused by GDP-L-fucose deficiency (GFUS mutation) in a child with developmental disability, growth retardation and dysmorphic facial features. GFUS protein and GDP-L-fucose were reduced leading to glycoprotein hypofucosylation. Moreover, the study shows that the conversion of GDP-D-[2- 3H]mannose to GDP-L-[2- 3H]fucose was dramatically reduced in patient-derived fibroblasts. Fucosylation was restored using retroviral complementation with the wildtype GFUS-cDNA of patient-derived fibroblasts which ascertained the causative effects of the variants detected.
The study well describes the effect of L-fucose supplementation on protein fucosylation in vitro and in vivo.

First we want to thank all of the reviewers for their helpful comments and suggestions to improve the manuscript.

Referee #1 (Remarks for Author):

The study described a novel CDG caused by GDP-L-fucose deficiency (GFUS mutation) in a child with developmental disability, growth retardation and dysmorphic facial features. GFUS protein and GDP-L-fucose in patient-fibroblasts were reduced. Moreover, the study shows that the conversion of GDP-D-[2- 3H]mannose to GDP-L-[2- 3H]fucose was also dramatically reduced in patient-derived fibroblasts. Fucosylation was restored using retroviral complementation with the wildtype GFUS-cDNA of patient-derived fibroblasts which ascertained the causative effects of the variants detected.-
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Fucose supplementation restored fucosylation in patient-derived fibroblasts stained with AAL lectin.
The study interestingly shows that oral L-fucose supplementation was followed by improved fucosylation of proteins and some clinical improvements. Overall the study harbors a considerable novelty in the field and deserves particular interest in the light of the explored therapeutic approach.

Revisions required
1. The introduction briefly reports some essential elements of glycobiology with particular regard to the biosynthesis of GDP-L-fucose which improves comprehension to the non-specialist readers. However, information on CDG first-line screening test (i.e. serum transferrin IEF) and related pitfalls is particularly envisaged at this point. Actually, this novel CDG adds to the list of CDG with normal serum transferrin IEF.

- *We agree to this important point. Due to didactical reasons, we preferred to point this diagnostic issue out in the discussion part. We added the sentence 'Thus, GFUS-CDG adds to the list of glycosylation defects with normal serum transferrin IEF.' To the penultimate paragraph of the discussion on page 14.*

2. When citing therapeutic strategies in known CDG, it would be interesting to briefly report the different circumstances that make useful some monosaccharides as potential treatments in CDG.

- *We added the sentence 'Those therapies usually rely on a surplus of the respective donor substrate or co-factor for the defective protein.' In the introduction in the second last paragraph on page 4*

3. Patient description. I understand that reported prenatal information are related to fetal (child?) growth and fetal movements.

- *Definition of "cerebral cysts" detected prenatally is vague and not sufficiently documented (i.e. localization, evolution during pregnancy are some information that should be added). -- Additional information on post-natal brain appearance as detected by newborn cerebral ultrasound might be meaningful also in the light of the increased head circumference at birth (97th pc).*

4. As the child was affected with a neurodevelopmental disorder, some information on possible seizure disorder and patient electroclinical features should be reported in the patient clinical description.

- *According to the reviewer suggestion we included some information about the mentioned issues in the clinical description.*

5. Biochemical and molecular investigations. The mRNA expression levels for the Golgi GDP fucose transporter SLC35C1 (54.4% {plus minus} 6.5%; p=***0.00003) as well as for the potential ER GDP-fucose transporter SLC35C2 (63.3% {plus minus} 17.4%; p=*0.035 (Fig.4B)) were significantly diminished. Is there any explanation for this finding? This is particularly important to address the use of sugar supplementation as well.

- *We rate this as a compensatory effect due to a lack of the substrate, GDP-L-fucose, for both transporters. We stated this in the discussion on page 12 paragraph 7.*

6. The effect of L-fucose supplementation on O-fucosylation is not adequately described in the results section. Please underline the strategies used to detect baseline deficiency and post-fucose modification of O-fucosylated proteins.

- *O-fucosylation depends on a sufficient supply of GDP-L-fucose, therefore we measured the expression of POFUT1/2 on the transcript and protein level and included both datasets in the results and discussion parts. Furthermore, we used secretion of ADAMTS13 in the serum as an indirect marker for protein-O-fucosylation by POFUT2. In short, we found a reduced POFUT2 and ADAMTS13 protein amount. As secretion of ADAMTS13 depends on the O-fucosylation of its thrombospondin repeats catalyzed by POFUT2, we conclude that the diminished GDP-L-fucose level first led to reduced POFUT2 and subsequently impacts fucosylation and thereby secretion of ADAMTS13.*
  The information can be found in the results part 'GFUS deficiency affects expression of downstream proteins associated with fucose metabolism' on page 8 and in the discussion

on pages 12/13 paragraph 9. Also expanded view figure 2 was updated with those data including statistics and an increased amount of control samples.

7. Clinical course after 19 months of oral L-fucose supplementation. The clinical course was monitored by growth parameters and standardized psychological tests. It would be beneficial to know if CDG severity scoring was evaluated before and during treatment (i.e. Nijmegen CDG rating scale).

- *see reply to 8. below.*

8. Although there was a considerable improvement in mental capacities on psychological tests, it would be interesting to add some information on modification of age-related activities of daily living, if any.

- *We have added the Nijmegen Pediatric CDG Rating Scales to our manuscript (abstract, case description, supplementary data) and have provided more details on the activities of daily living to the follow up section.*

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

1. Regarding the TECHNICAL QUALITY, my major concerns are about the xCGE-LIF analysis of N-glycome and FACS analysis of fucosylation of PBMCs. For these two points, although the presented data are finally rather convincing, corresponding (supplemental) Figure legends are overall unclear and very difficult to understand.

- *According to the reviewer suggestion we included more detailed figure legends for the xCGE LIF analysis and FACS analysis.*

More precisely,
For xCGE-LIF,
- the number of controls (n=2) is unsatisfactory.
- thus, there is no corresponding statistical evaluation of results.

- *We increased the number of controls to n=10. SD for the controls was added to the result part 'Normal behaviour of serum transferrin during isoelectric focusing but abnormal whole serum N-glycosylation'. Expanded view figure 1 was adapted as well to increase clarity.*

- nomenclature of N-glycans (A? G? D?...) should be clarified

- *The respective information was added to the figure legend of expanded view figure 1 (N-glycan structures were produced using Glycan Builder 2 (Tsuchiya, Aoki et al., 2017) following the Symbol Nomenclature for Glycans (SNFG) guidelines (Neelamegham, Aoki-Kinoshita et al., 2019). Naming of N-glycan structures was adapted from the Oxford nomenclature (Doherty, Theodoratou et al., 2018)).*

- the claimed shift in the relative composition of the N-glycome is unclear viewing the 'difficult' Table EV1 (and not 'supplementary table 2' as mentioned?)

- *Regarding the N-glycome composition, Data presentation of the results was reevaluated and can be found in a clarified version of expanded view figure 1 and 'Expanded View Table 1: N-Glycans identified and quantified by xCGE-LIF'.*

- why the announced increase of hybrid-type N-glycans not linked to detectable transferrin glycosylation defects?

- *Transferrin displays the gold marker in CDG diagnostics. Unfortunately, it is not a valuable tool for detection of hybrid type glycans as the overwhelming part of N-glycans bound to transferrin belong to the complex type. Hybrid glycans make up only about 4-5% and thus have a rather small influence on the transferrin glycosylation status.*

For FACS analysis of the fucosylation of PBMCs,
- references to figure 5 and to suppl Fig3 (3 pages) seem wrong and are confusing. For example, fig 5C (8 weeks fucose) is mentioned for 2 weeks fucose in the Fig legend?

- *We corrected and modified figure legend 5. We are sorry we were not able to figure out what is wrong with 8 weeks and 2 weeks.*

- Legend of Fig EV3 is very unclear

- *We modified the legends for the FACS part for figure legend EV3.*

In addition,
FigEV4 is never mentioned in the manuscript? Is there a missing page in the submitted text?
We included Fig EV4 in the text.

- *A reference to FigEV4 was included.*

Regarding the Fig. 5A (AAL lectin blot), I'm surprised that only 3 protein bands (what MW?) seem fucosylated in serum... Furthermore, legends of fig 5B/5C should be clarified.

- *Concerning the AAL blot of control and patient serum (Fig. 5A), we increased brightness and contrast to clarify that there are more protein bands than three.*
- *We modified figure legend 5.*

NOVELTY is high since Feichtinger and col. described here a new treatable CDG.

MEDICAL IMPACT could be rather high (medium?) for this probably treatable CDG that concerns very few affected individuals. But evaluated clinical test(s) are rather scarce with rather slight improvements...

MODEL SYSTEM (fibroblasts, serum, platelets, PBMCs and the affected girl) are adequate.

Concerning the text of the manuscript (essentially Discussion):
- I feel that the reader could be very confused between O-fucosylation and fucosylation of O-glycans (e.g. GalNAc O-glycans).

- *We removed this inconsistency.*

- Bombay group should be further explained. Unclear point.

- *We added this sentence to the manuscript: People who carry no functional H antigens on their red blood cells (e.g. due to hypofucosylation), present serologically with the Bombay (0h) blood group.*

- 'expression of SLC35C1 and FUT8 was found to be reduced in QRT-PCR studies' : where are the related data for FUT8? I did not find.

- *Expression data for SLC35C1, SLC35C2, FUT8, POFUT1 and POFUT2 were included in the results and discussion part and in figure 4 and expanded view figure 2. Please also see our answer to reviewer 1 above*

- Why the accumulation of FA3G3S1(2,6) (??) is interesting?

- *By using an increased number of controls in combination with more exoglycosidases, we were able to decrease the number of undistinguishable N-glycan structures, which led to the identification of a specific core-fucosylated glycan (FA2G2S1(2,6)S1(2,3), which might be interesting as it could be used as a biomarker for GFUS-CDG diagnosis.*

- 'expression of POFUT1 and POFUT2 were normal in qRT-PCR studies': where are the related data? I did not find. – *please see above*
- what about the described elevated protein level of FUT8?

- *Please see above*

MINOR POINTS:
- 'Kaufmann ABC' is unclear in the abstract.

- *The Kaufmann ABC is a standardised and world-wide widely used test battery. It* helps to identify an individual's strengths and weaknesses in cognitive ability and mental processing. The information provided by the KABC-II can facilitate clinical and educational planning, treatment planning and placement decisions. The internal consistency reliability coefficient for core and supplementary subtests demonstrate the KABC-II has good reliability. We would therefore think that this does not need more clarification.

- GAG (O-xyl) should be mentioned in the introduction.

- *o-xyl was added to the first paragraph of the introduction*

- 'From birth on lack of appetite' (case reports): I don't understand.

- *She did not indicate when she was hungry, neither with crying for breastfeeding in the first months, neither by showing any interest in food later on. This improved after the start of L-fucose and is now detailed more precisely in the follow up section.*

- what were the tested prediction programs for gene variant pathogenicity?

- *We added SIFT, PolyPhen, REVEL, MetaLR to the manuscript (see Results 'Biallelic variants in GFUS', page 5/6).*

- how long for 100 µM L-fucose treatment? (results).

- *L-fucose treatment was done for 48 h and this information was added to the method- and results part.*

- ADAMTS13 should be clarified in the results part: ADAMTS13, a heavily O-fucosylated protein.

- *We added this information to the respective paragraph 'GFUS deficiency affects expression of downstream proteins associated with fucose metabolism' in the results.*

- 'This could be due to the functional redundancy of the salvage and the de novo pathway': unclear.

- *We replaced `redundancy´ by `overlap´ to be more precise on the meaning of the sentence.*

Referee #3 (Remarks for Author):

This is a well-written manuscript on a novel CDG.
I have no problem with the content. The following are a few small corrections:
ABSTRACT
L 4: delay, mild

- *Corrected.*

L 5: synthase,

- *Corrected.*

L 8: serum glycoproteins, leukocytes

- *Corrected.*

L 12: serum glycoproteins

- *Corrected.*

INTRODUCTION

L 1: Some 2 %

- *Corrected.*

L 6: anchored protein synthesis

- *Corrected.*

L 11, 12: guanosine (not guanidine!)

- *Corrected.*

L 23: CDG (is singular as well as plural)

- *Corrected.*

10th last sentence: CDG no therapeutic

- *Corrected.*

RESULTS
P 2, L 17: GDP-D-mannose

- *Corrected.*

eight last line: no side effects

- *Corrected.*

Referee #4 (Remarks for Author):

The manuscript details the identification of compound heterozygous missense mutations in GFUS encoding GDP-L-fucose synthase. This led to a novel CDG. The authors also show that fucose supplementation was at least partially effective as a treatment. The manuscript is well written and concise. The two aspects of the work, namely i) the identification of a new CDG, GFUS-CDG, and ii) the clinical study on the effectiveness of treatment with fucose are valuable to the scientific and clinical community.

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Major comments:

• For several data panels and results it seems only one control was used to compare with that of the patient (e.g. EV Fig 2.) Ideally two or several controls would be used for comparison, particularly for an inherently variable method such as western blotting, and bearing in mind only one patient was available so statistical analysis was already very difficult. If the authors in fact used several controls and the data shown was purely for presentation reasons, it should be stated clearly in the text. Particularly with regards to EV Fig 2, this makes it very hard to draw conclusions from the comparison of a single control with a single patient. This should at the very least be noted.

- *We added more controls to the experiments shown in EV Fig 2 and EV Fig 1 and also included the data to the results and discussion part. Please see also our comments to reviewer #1.*

Minor comments:

• Abstract/introduction: The type of variant identified in the patients is not specified beyond 'biallelic' before the results section. The authors should add that the variants are compound heterozygous missense mutations to the abstract and/or introduction.

- *We included the required information in the abstract line 4: "…* we detected biallelic variants in *GFUS* (NM_003313.4) c.[632G>A];[659C>T] (p.[Gly211Glu];[Ser220Leu]) in a patient presenting with global developmental delay …"

• In the discussion it is mentioned that when measured by qPCR, lower levels of transcripts from FUT8 were found. Firstly, this is not mentioned in results and should be added. Secondly, does this not oppose the higher FUT8 protein levels found by western blot? Can you explain this discrepancy?

- *We thank the reviewer to point us to a mistake made in our manuscript: The transcript and protein level of FUT8 were both found to be increased in case of the patient. We apologize for this confusion. The error was corrected and classified accordingly in the discussion (see Discussion paragraph 8, page 11).*

• The authors also present qPCR data showing that GFUS, SLC35C1, SLC35C2 were all downregulated on a transcriptional level and describe this as a possible compensatory mechanism. Is there evidence of this in other defects of the fucose metabolic pathway?

- *To the best of our knowledge no qPCR data on GFUS and the transporters have been published so far.*

• Do the authors think that in this case, with some residual enzymatic activity of GFUS, there is potential for supplementation with mannose (or analogue) as a substrate therapy? Or would this be unlikely to improve on the results already achieved by fucose therapy?

- *Thanks for this question, which is not only of interest of the biochemical but as well of the financial point of view, as the costs for mannose are about 1000€ per kg and for fucose are about 7000€ per kg. Regrettably, measurements of nucleotide activated sugars in patient derived fibroblasts (data not shown) already showed an increase in GDP-mannose compared to control cells, which led us to the assumption that further supplementation of mannose would have no additional effect as a substrate therapy.*

• Park et al. (https://pubmed.ncbi.nlm.nih.gov/33312876/) recently reported that fucose treatment was partially successful in a case of FUT8-CDG. Although their study was only on two patients and showed very tentative results, it is very relevant to this manuscript and should be at least mentioned.

- *The publication of Park et al. on FUT8-CDG is included in the discussion on page 10.*

1st Revision - Editorial Decision 21st Jul 2021

21st Jul 2021

Dear Dr. Wortmann,

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. We have now received the enclosed reports from the two referees who agreed to re-assess it. As you will see the referees are now supportive and I am pleased to inform you that we will be able to accept your manuscript pending the following amendments:

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

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

The current version of the manuscript has carefully taken into account all my previous revisions. The revised manuscript is clear and complete and it deserves to be published in EMBO Molecular Medicine in the opinion of this reviewer.

Referee #1 (Remarks for Author):

The current version of the manuscript has carefully taken into account all my previous revisions. The revised manuscript is clear and complete. I have not further comments for the present study.

Referee #2 (Remarks for Author):

All my comments and corrections have been fulfilled.
Nice, clear, and very interesting work!
Thank you!

The authors performed the requested editorial changes.

2nd Revision - Editorial Decision 28th Jul 2021

28th Jul 2021

Dear Dr. Wortmann,

We are pleased to inform you that your manuscript is accepted for publication and is now being sent to our publisher to be included in the next available issue of EMBO Molecular Medicine.

We would like to remind you that as part of the EMBO Publications transparent editorial process initiative, EMBO Molecular Medicine will publish a Review Process File online to accompany accepted manuscripts. If you do NOT want the file to be published or would like to exclude figures, please immediately inform the editorial office via e-mail.

Please read below for additional IMPORTANT information regarding your article, its publication and the production process.

Congratulations on your interesting work,

Kind regards,
Jingyi

Jingyi Hou
Editor
EMBO Molecular Medicine

*** *** *** IMPORTANT INFORMATION *** *** ***

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Corresponding Author Name: Saskia B. Wortmann
Journal Submitted to: EMBO Mol Med
Manuscript Number: EMM-2021-14332-V2

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

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.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

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?	To truly reflect the population, sample size was chosen to be as large as possible for the respective analysis. Of course, this also depends on the availability of serum samples from the patient.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA
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.	NA
For animal studies, include a statement about randomization even if no randomization was used.	NA
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.	Samples were labeled with pseudonyms during their measurements due to data protection guidelines in Europe, so that no assignment is possible during the analysis. Cooperation partners only had access to the pseudonyms and could not actively assign the samples.
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	For all statistical tests, either one-way ANOVA with the assumption of a Gaussian distribiution of residuals, or unpaired t-tests with Individual variance for each row and an alpha of 0.05 according to the Holm-Šídák method were used.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	To prove the normal distribution, the Shapiro-Wilk test was used.
Is there an estimate of variation within each group of data?	Whenever it was possible, the estimate of variance is given. In individual experiments, the serum of the patient was measured, of which there are no biological replicates, as this patient is unique.

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http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repor

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http://ClinicalTrials.gov
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Is the variance similar between the groups that are being statistically compared?	It was assumed that each group has individual variances.

C- Reagents

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 (see link list at top right), 1DegreeBio (see link list at top right).	Catalog numbers of antibodies used can be found in the Methods part.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	For the analyses commercially available control cell lines (www.atcc.org) were used and compared to patient-derived fibroblasts. Tests for contamination by mycoplasms were performed regularly.

* 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.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (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 (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	Ethics Committee Salzburg (415-E/2552/10-2019)
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 is stated in the Methods part.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	This is stated in the Methods part.
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for '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	NA
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G- Dual use research of concern

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