OPEN PEER REVIEW

Open Peer Review (OPR)

As a new feature for progressing towards transparency, we decided to open a new window for all of our editors as well as authors titled "**Open Peer Review**". We hope by this new facility, our reviewers will be more motivated and authors will be more satisfied with the review process. We believe that publishing our peer review reports could make a transparent and clear environment for all our efforts within a journal, but not all reviewers tend to publish their comments.

What is "Open Peer Review" process?

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Advantages of "Open Peer Review" process

- More transparency, constructiveness and tactful comments of the peer review process: leads to an increase in the quality of reviews
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- Authors' satisfactions from the review process: Increases honesty between authors and reviewers
- Education of both authors and new students
- Prevents reviewers from following their individual agendas and leading to the detection of reviewers' conflicts of interests

You can find out more at:
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Hepatitis Monthly
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Peer Review Report for "Biochemical and Bioinformatic Characterization of Patients with a Sodium Taurocholate Cotransporting Polypeptide Mutation"

Author(s): Xuan Li, XueQian Zhou, Hong Yang, LiangJun Zhang, XiaoXun Zhang, Jin Chai

Review Timeline:

Submit Date: 12 Dec 2021
Revised Date: 23 Jul 2022
Accepted Date: 23 Jul 2022

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

Raika Jamali: Reviewer | Revision (0)

23 Dec 2021

Dear Professor Alavian,
Thank you for sending me the article entitled “Clinical and bioinformatic characterization of patients with sodium taurocholate co-transporting polypeptide (NTCP) mutation” for review. This single center, cross sectional study evaluated the clinical and laboratory assessments (including liver function, thyroid function, lipid profile, and vitamin D) in 12 patients with NCTP mutation. All homozygotes individuals in this study had normal levels of blood lipids, thyroid hormones and vitamin D. Except for p.Ser267Phe, multiple gene mutations have found in 5 homozygous and might affect the level of bile acids, but p.Ser267Phe in NTCP still to be the most important reason result in hypercholanemia. The authors concluded that p.Ser267Phe mutation has no clinical deleterious effects in both children and adults. There are some comments as the followings:
1-According to the title (Clinical and….), the clinical data should be added to the manuscript. Only age and gender are provided herein.

2-One important biochemical parameter for evaluation of hepatocyte secretary function is gamma-glutamyl transferase (GGT). This biomarker is not evaluated in this study. This limitation should be addressed in the discussion.
3-There are conditions that influence the bile acid pool. These conditions should be evaluated in the study subjects. For example malabsorbtive diseases, cholestatic liver diseases and medications could alter the enterohepatic circulation of bile acids. These are confounding factors while assessing the value of bile acids in subjects. I believe this shortage should also be explained in the discussion.
4-Diabetes mellitus and insulin resistance are major factors that affect the lipid profile. These conditions should be evaluated in accompany to lipid profile assays.
5- The last word is consuming all the conditions that influence the laboratory investigations, while performing a genomic study.
6-The data in tables 2, 3, 4, and 5 could be merged in one table. Please choose an appropriate title for the new table that showing the comparisons of biochemical data in the study groups.

REFEREE: Reviewer | **Revision** (0) 29 Dec 2021

Dear Editor-In-Chief
The manuscript investigates the clinical and genetic characterization of hypercholanemia patients carrying Ser267Phe mutation in the NTCP gene. The authors used whole-genome and Sanger sequencing to identify such a mutation in 12 individuals in terms of homozygous, heterozygous, and wild type. After that, they tried to find a correlation or difference between biochemical data and/or SNPs in genes involved in bile acid and cholesterol metabolism among the individuals. Unfortunately, the number of studied genomes is very few for finding statistically significant positive or negative correlations either in clinical data or SNPs. Moreover, the biochemistry data analysis has not led to a novel finding between the presence and absence of the mutation (as stated by the authors on page 6 line 167). In the SNP analysis, no SNP statistical analysis has been used and the relationship between the five proposed SNPs and hypercholesterolemia has been postponed to further study. Therefore, I do not recommend the manuscript for publication in the journal unless resolving the mentioned concerns.

best regards,

The reviewer

REFEREE: Reviewer | **Revision** (0) 5 Jan 2022

1- Please write the novelty of your study compared to similar articles published in recent years on the Ser267Phe mutation and hyperchloremia.
2- what is the reason for using the whole genome sequencing method to confirm the existence of p. Ser267Phe mutation only (The rest of the genes are not mentioned in this article)? It seems that the sanger method was sufficient for this evaluation.
3- What is the reason for choosing SLC gene?
4- Please add the statistical program used in this study in the “statistical analyses “section
5- Please add the details of the platform that was used in this project including read length,

average coverage….
6- Please add references of GATK tool, Sam tools, NGS QC toolkit,ANNOVAR…
7- Please add version and reference of picard tool
8- Is the SLC10A1 gene obtained from databases such as OMIM, Clinvar,Doaf,…. ??
9- Please add the full name and version of some tools .for example: SIFT, PolyPhen2
10- Please examine the conservancy of variants using multiple sequence alignment and also evaluate three-dimensional structures of both wild-type and the candidate variant and visualize any changes in protein folding and structure.
11- Pease add threshold of classification of variants with sift (deleterious or tolerated) and PolyPhen2

REFEREE: Associate Editor | **Revision** (0) 9 Jan 2022

Dear EIC,

Manuscript # 121842 entitled, " Clinical and bioinformatic characterization of patients with sodium taurocholate co-transporting polypeptide (NTCP) mutation" was evaluated by 3 expert reviewers. According to the reviewers there are several points that should be exactly addressed in a revised version of the manuscript (based on the raised comments), before re-evaluation of the manuscript to reach a final decision could be achieved.

Seyed Moayed Alavian: EIC | **Revision** (0) 9 Jan 2022

Dear Authors,

Thank you for your submission. Please follow the peer-review comments and submit a revision.

Regards,
EIC

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

We appreciate the reviewer' precious comments and advice. Those comments are all valuable and very helpful for revising and improving our paper. We have revised the manuscript accordingly，and our point-by-point responses are presented below.

For Reviewer 1:

1- **Q:** Please write the novelty of your study compared to similar articles published in recent years on the Ser267Phe mutation and hyperchloremia.

R: Thank you very much for your comment. Despite the conclusions of this paper are the same as previous study, we still try to make up for the following two shortcomings. In this study, biochemical description of recruited individuals was done to enrich the characterization of p.Ser267Phe in NTCP, which is more comprehensive than other articles. Until then, whether the Ser267Phe mutation is the only reason influencing bile acids in patient still unclear. To confirm if there are any mutations co-exist with Ser267Phe mutant influence the high levels of bile acids, we performed whole-genome sequencing on all recruited individuals. We supported a reliable result in this paper, which showed that although the Ser267Phe mutation is the most important reason related to the level of bile acids, there are some mutations might improve susceptibility of hypercholanemia.

2- **Q:** What is the reason for using the whole genome sequencing method to confirm the existence of p. Ser267Phe mutation only (The rest of the genes are not mentioned in this article)? It seems that the sanger method was sufficient for this evaluation.

R: Thanks for the professional comment . We realized that we might not have been clearly described these issues adequately of our study in the previous manuscript. We have added a more detailed interpretation in the "discussion part". In our study, we performed whole genome sequencing test on a series of patients with unexplained hyperbilirubinemia at first. And the mutation of NTCP p.Ser267Phe were found in some patients. Sanger sequencing was used to further validate this mutation. In addition, the whole genome sequencing results indicated that although the Ser267Phe mutation is the most important reason related to the level of bile acids, there are some mutations (such as rs2287622 in ABCB11, rs927344 in ABCC2, rs679899 in APOB, rs562556 in PSCK9, and rs11073964 in VPS33B.) might improve susceptibility of hypercholanemia. More details have been provided in sections of "result" （*The whole-genome sequencing excludes the involvement of other BA metabolism-associated genes.*） and "discussion" to describe this question.

3- What is the reason for choosing SLC gene?

Thank you for your comment and we apologize for the logic problems in the original manuscript. Firstly, we observed a series of patients with unexplained hypercholanemia. Next, we performed whole genome sequencing test on these patients, which revealed that some of them had a mutation in the SLC10A1 gene (*NTCP p.Ser267Phe*). The SLC10A1 gene encodes the protein of NTCP which is very important to bile acid transport. According to present reports, mutation of NTCP p.Ser267Phe probably be related to hypercholanemia. However, research between the mutation and hypercholanemia is not enough and more studies are needed. Therefore, we chosen patients with NTCP p.Ser267Phe mutation for further study.

4- **Q:** Please add the statistical program used in this study in the "statistical analyses "section

R: Thank you for your suggestion. The data were analyzed by SPSS version 23.0 for windows (SPSS Inc.,Chicago,IL, USA) and we have added the statistical program in the "statistical

analyses “section.

5- **Q:** Please add the details of the platform that was used in this project including read length, average coverage….

R: Thank you for your suggestion. The platform we used in this project is the Illumina sequencing platform (HiSeq X Ten platform, Illumina Inc., San Diego, CA, USA). The read length is 350bp and average coverage is 29.34X. We have added the suggested content to the “method” section.

6- **Q:** Please add references of GATK tool, Sam tools, NGSQC toolkit, ANNOVAR…

R: Thank you for your suggestion. References of those tools we used in WGS have been list below:

- NGSQC toolkit: Dai M, Thompson R C, Maher C, et al. NGSQC: cross-platform quality analysis pipeline for deep sequencing data[J]. BMC Genomics, 2010, 11 Suppl 4(4):S7. (https://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-11-S4-S7)
- Sam tools: Li H, Handsaker B, Wysoker A, et al. The Sequence Alignment/Map (SAM) Format and SAMtools[J]. Transplantation Proceedings, 2009, 19(1 Pt 2):1653-4.
- BWA: Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform.[J]. Bioinformatics, 2010, 25(5):1754-1760.
- GATK tool: McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M and DePristo MA (2010) The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Research, 20:1297-303.
- ANNOVAR: Wang K, Li M, Hakonarson H. (1).[J]. Nucleic Acids Research, 2010, 38(16):e164.

Also, we have added these references to the “method” section.

7- **Q:** Please add version and reference of picard tool.

R: Thank you for your suggestion. The version of picard tool is v2.12.0. Currently there is no Picard paper. We cite Picard by the website:

Picard tools. Association of GitHub, Inc [updated 2017 Oct 2; cited 2017 Oct 10]. Available from: https://broadinstitute.github.io/picard/.

We have added the references to the “method” section.

8- **Q:** Is the SLC10A1 gene obtained from databases such as OMIM, Clinvar,Doaf,…. ?

R: Thank you for your question. The SLC10A1 gene obtained from the National Center for Biotechnology Information database. We have added this information into the “method” section.

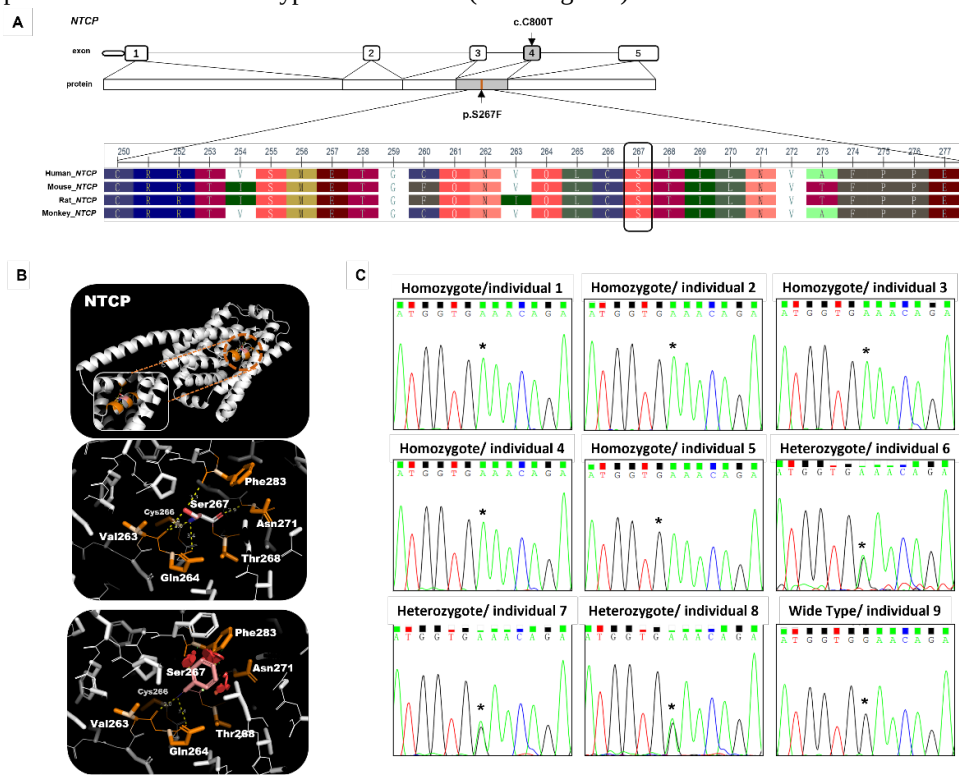
9- **Q:** Please add the full name and version of some tools .for example: SIFT, PolyPhen2

R: We deeply appreciate the reviewer’s suggestion. The full name and of SIFT is Sorting Tolerant From Intolerant (dbNSFP version 3.3a), and the full name and of PolyPhen2 is Polymorphism Phenotyping v2 (version 2.2). We have added these references to the “method” section.

10- **Q:** Please examine the conservancy of variants using multiple sequence alignment and also evaluate three-dimensional structures of both wild-type and the candidate variant and visualize any changes in protein folding and structure.

R: We are grateful for the suggestion. To be more clearly and in accordance with the reviewer concerns, we have added multiple sequence alignment and three-dimensional structures

prediction of NTCP wild type and mutation (see in figure1).



11- **Q:** Pease add threshold of classification of variants with sift (deleterious or tolerated) and PolyPhen2.

R: Thank you for the suggestion. We have added the information of classification of variants with SIFT and PolyPhen2 in the section of “methods”. The protein function prediction of SIFT is based on SIFT score which calculated by sequence homology and the physical/chemical similarity between amino acids (Deleterious means sift<=0.05, Tolerated means sift>0.05). On the other hand, we used Polyphen2_HDIV_score for protein function prediction, which is based on the HumanDiv database for complex diseases (Probably damaging : score>=0.957, possibly damaging : score=0.453 ~ 0.956; benign: score<=0.452).;

For Reviewer 2:

Dear Editor-In-Chief

The manuscript investigates the clinical and genetic characterization of hypercholanemia patients carrying Ser267Phe mutation in the NTCP gene. The authors used whole-genome and Sanger sequencing to identify such a mutation in 12 individuals in terms of homozygous, heterozygous, and wild type. After that, they tried to find a correlation or difference between biochemical data and/or SNPs in genes involved in bile acid and cholesterol metabolism among the individuals. Unfortunately, the number of studied genomes is very few for finding statistically significant positive or negative correlations either in clinical data or SNPs. Moreover, the biochemistry data analysis has not led to a novel finding between the presence and absence of the mutation (as stated by the authors on page 6 line 167). In the SNP analysis, no SNP statistical analysis has been used and the relationship between the five proposed SNPs and hypercholesterolemia has been postponed to further study. Therefore, I do not recommend the manuscript for publication in the

journal unless resolving the mentioned concerns.
best regards,

R: Thank you for your careful review. 1). The mutation frequency of NTCP p.Ser267Phe in East Asia is reported to be between 2~10% which means great clinical significance. However, study about patients with hyperuricemia caused by NTCP p.Ser267Phe mutation is not enough. In this study, We provided a comprehensive biochemical index information of patient. Although there are a lot of negative results in our study as you pointed out, it does helps researchers further understand this mutation. 2) We performed whole genome sequencing on individuals included in this study. According to references and database prediction, we have identified five SNP which might increase the susceptibility to NTCP S267F mutation induced hypercholanemia. However, the number of recruited members is limited and statistics like correlation analysis is unsuitable. This is indeed the weak point of our article. The relationship between these five sites and NTCP p.Ser267Phe induced hypercholanemia required to construct numerous cellular and animal models. In the future, we will research on this topic and hope you may understand our difficulty at this stage.

For Reviewer 3:

Dear Professor Alavian,

Thank you for sending me the article entitled “Clinical and bioinformatic characterization of patients with sodium taurocholate co-transporting polypeptide (NTCP) mutation” for review. This single center, cross sectional study evaluated the clinical and laboratory assessments (including liver function, thyroid function, lipid profile, and vitamin D) in 12 patients with NCTP mutation. All homozygotes in this study had normal levels of blood lipids, thyroid hormones, and vitamin D. Except for p.Ser267Phe, multiple gene mutations have found in 5 homozygous and might affect the level of bile acids, but p.Ser267Phe in NTCP still to be the most important reason result in hypercholanemia. The authors concluded that p.Ser267Phe mutation has no clinical deleterious effects in both children and adults. There are some comments as the followings.

1- **Q:** According to the title (Clinical and….), the clinical data should be added to the manuscript. Only age and gender are provided herein.

   R: Thank you for the detailed review. We provided all the clinical information that we gathered from recruited individuals in this study. We are sorry for the inappropriate title of this paper. In the revised version, the title has been modified to “**biochemical and bioinformatic characterization of patients with sodium taurocholate co-transporting polypeptide (NTCP) mutation**”. We appreciate your elaborate efforts in reviewing. Thank you very much!

2- **Q:** One important biochemical parameter for evaluation of hepatocyte secretary function is gamma-glutamyl transferase (GGT). This biomarker is not evaluated in this study. This limitation should be addressed in the discussion.

   R: Thank you for your precious comments and advice. We apologize for this unprofessional mistake. The gamma-glutamyl transferase data has been added in table 2 now.

Table2. Biochemical Parameters of the Study Cohort Grouped by Genotype

Genotype (rs2296651)			Wild Type (GG) (n=4)	Heterozygous (GA) (n=3)	Homozygous (AA) (n=5)
Liver/Renal Function					
Test	Unit	Ref.			
ALT	0-42	(IU/L)	14.87±2.76	21.00±18.25	20.00±11.77
AST	0-42	(IU/L)	17.70 (17.65, 23.35)	16.00 (15.50, 21.50)	30.00 (26.00, 42.00)
ALP	34-114	(IU/L)	192.33±199.95	59.33±32.32	190.20±90.73
GGT	5-50	(IU/L)	13.67±3.21	20.67±9.07	20.67±7.20
TBA	0-10	(umol/L)	2.80 (2.25, 3.20)	4.90 (3.40, 5.95)	35.20 (34.00, 47.00) *
TBIL	6-21	(umol/L)	12.70±3.93	11.03±1.37	6.82±2.17 *

3- **Q:** There are conditions that influence the bile acid pool. These conditions should be evaluated in the study subjects. For example, malabsorbtive diseases, cholestatic liver diseases and medications could alter the enterohepatic circulation of bile acids. These are confounding factors while assessing the value of bile acids in subjects. I believe this shortage should also be explained in the discussion.

R: We are grateful for the suggestion and try to discuss this shortage in the revised manuscript . We understand there are many factors that affect the bile acid pool, which needs to be fully excluded before we diagnose *NTCP p.S267F* mutation induced hypercholanemia. Not did we find any members with symptoms of malabsorbtive diseases or cholestatic liver diseases and have long term medications. We have tried our best to exclude those factors withing a detailed history taking and physical examination. But due to limited conditions, we did not conduct detailed assist examination. In the future, we will focus on this shortage and hope you may understand our difficulty at this stage.

4- **Q:** Diabetes mellitus and insulin resistance are major factors that affect the lipid profile. These conditions should be evaluated in accompany to lipid profile assays.

R: Thank you very much for your suggestion. We totally agree with your opinion. Indeed, there are some shortages in our research. We did not evaluate diabetes mellitus and insulin resistance in patients because of there are no symptoms of diabetes mellitus and insulin resistance in physical examination of patients. In this study, we detected the blood lipid level of the patient, although indicators like total cholesterol, triglyceride, high-density lipoprotein-cholesterol, and low-density lipoprotein-cholesterol cannot fully assess the blood lipid level of the patient, it still affords a relatively evaluation that lipid metabolism of these members is basically normal. Thank you again for your precious suggestion and we will more rigorous in the future.

5- **Q:** The last word is consuming all the conditions that influence the laboratory investigations, while performing a genomic study.

R: Thank you very much. If we understand correctly, this suggestion is asking why we using the whole genome sequencing method to only study the *p.Ser267Phe* mutation. In fact, we collected number of hypercholanemia patients with unknown reason. According to the results of WGS, patients with *NTCP p. Ser267Phe* mutation only took a limited part. In addition, the results indicated that although the Ser267Phe mutation is the most important reason related to the level of bile acids which might improve susceptibility of hypercholanemia. Also, we are devoted to focus on other hypercholanemia patients with different gene mutations.

6- **Q:** The data in tables 2, 3, 4, and 5 could be merged in one table. Please choose an appropriate title for the new table that showing the comparisons of biochemical data in the study groups.

R: Thank you for the suggestion. Table 2 to 5 have been merged in one table (Table2).

Table2. Biochemical Parameters of the Study Cohort Grouped by Genotype

Genotype (rs2296651)			Wild Type (GG) (n=4)	Heterozygous (GA) (n=3)	Homozygous (AA) (n=5)
Liver/Renal Function					
Test	**Unit**	**Ref.**			
ALT	0-42	(IU/L)	14.87±2.76	21.00±18.25	20.00±11.77
AST	0-42	(IU/L)	17.70 (17.65, 23.35)	16.00 (15.50, 21.50)	30.00 (26.00, 42.00)
ALP	34-114	(IU/L)	192.33±199.95	59.33±32.32	190.20±90.73
GGT	5-50	(IU/L)	13.67±3.21	20.67±9.07	20.67±7.20
TBA	0-10	(umol/L)	2.80 (2.25, 3.20)	4.90 (3.40, 5.95)	35.20 (34.00, 47.00) *
TBIL	6-21	(umol/L)	12.70±3.93	11.03±1.37	6.82±2.17 *
DBIL	0-6	(umol/L)	2.57±0.67	4.50±1.84	2.98±0.89
TP	66-83	(g/L)	72.83±0.90	72.30±1.93	70.40±5.69
Alb	38-51	(g/L)	46.60 (45.40, 47.20)	45.20 (45.0, 46.6)	49.00 (48.80, 49.00)
G	25-38	(g/L)	26.63±2.72	24.00±5.19	20.84±4.09
A/G	1.2-2.5		1.76±0.25	1.77±0.17 *	2.45±0.49 *
UA	155-428	(umol/L)	374.67±124.64	286.33±138.60	277.80±87.42
Urea	1.7-8.3	(mmol/L)	3.97±1.07	3.81±0.63	3.12±1.26
Cre	45-84	(umol/L)	61.40 (46.20, 61.6)	43.00 (42.50, 57.35)	36.00 (21.00, 38.00)
Blood Fat					
TCH	3.1-5.7	(mmol/L)	4.63±0.30	4.00±1.14	4.16±0.90
TG	0.4-1.73	(mmol/L)	1.28 (1.02, 2.185)	0.76 (0.53, 1.56)	1.07 (0.78, 1.43)
HDL-C	0.9-2	(mmol/L)	1.49±0.52	1.44±0.39	1.52±0.31
LDL-C	2.07-3.1	(mmol/L)	2.73±0.28	2.36±0.81	2.64±0.91
Serum Thyroid Hormone					
T3	1.3-3.1	(nmol/L)	1.35 (1.30, 1.58)	1.70 (1.65, 1.76)	2.09 (1.86, 2.16)
T4	66-181	(nmol/L)	98.70 (94.41, 121.22)	91.43 (89.87, 94.35)	110.05 (104.74, 144.10)
FT3	3.1-6.8	(pmol/L)	2.97±0.70	2.57±0.09	3.28±0.52
FT4	12-22	(pmol/L)	15.82 (15.69, 16.55)	16.49 (14.96, 17.09)	21.49 (18.22, 23.09)
TSH	0.27-4.20	(ulU/ml)	0.78±0.33	1.01±0.39	0.56±0.19
Vitamin D					
Vitamin D(25-OH)	25-200	(pmol/L)	50.60 (47.18, 55.02)	45.06 (42.86, 47.55)	32.26 (30.60, 34.57)
Routine Blood Tests					
WBC	3.5-9.5	(10^9/L)	7.78±1.00	5.69±0.64	7.48±2.56
NEU	1.8-6.3	(10^9/L)	4.47±0.81	3.33±0.71	2.62±0.88 *
LYM	1.1-3.2	(10^9/L)	2.79±0.30	1.79±0.10*	3.97±2.74
MON	0.1-0.6	(10^9/L)	0.35±0.16	0.39±0.01	0.51±0.21
BAS	0-0.06	(10^9/L)	0.00 (0.00.0.11)	0.01 (0.005, 0.01)	0.02 (0.01, 0.03)
EOS	0.02-0.52	(10^9/L)	0.13 (0.09.0.21)	0.20 (0.12, 0.23)	0.15 (0.05, 0.17)
RBC	4.3-5.8	(10^{12}/L)	4.88±0.33	4.95±0.50	4.79±0.56
HGB	115-150	(g/L)	141.00±9.64	141.00±24.02	127.80±17.58
MCV	82-100	(%)	86.57±5.26	88.13±12.63	86.04±13.52
MCH	27-34	(pg)	28.93±1.87	28.43±4.87	26.92±3.71
MCHC	316-354	(g/L)	333.00 (333.00, 335.00)	318.00 (313.00, 331.00)	311.00 (309.00, 317.00)
RDW-SD	37-54	(%)	41.20±1.01	46.07±3.56 *	43.58±1.82
RDW-CV	11-16	(%)	13.00 (12.75, 13.50)	13.70 (13.25, 14.95)	14.10 (14.00, 14.40)
PLT	125-350	(10^9/L)	287.00±51.47	195.67±17.67 *	310.40±25.15 **
MPV	9-13	(fl)	9.70±0.26	9.47±1.02	9.66±0.79
PDW	9-17	(fl)	11.50±0.10	16.13±0.90 *	15.66±0.52 *

Values are means SD±SEM or Md (P25, P75); * P < 0.05 VS Wild type group, ** P < 0.05 versus Heterozygote group.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TBA, total bile acid; TBIL, total bilirubin; DBIL, direct bilirubin; TP, total protein; Alb, albumin; G, Globulin; UA, uric acid; Cre, creatinine; TCH, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; T3, triiodothyronine; T4, thyroxine, FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroid-stimulating hormone; WBC, white blood cell count; NEU, neutrophil; LYM, lymphocyte; MON, monocytes; BAS, Basophils; EOS, eosinophil; RBC, red blood cell count; HGB, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, RBC distribution Width; SD, standard deviation; CV, PLT, platelet; coefficient of variation; MPV, mean platelet volume; PDW, platelet distribution width;GG, the Wild Type for p.Ser267Phe in SLC10A1; GA, the Heterozygote for p.Ser267Phe in SLC10A1; AA, the Homozygote for p.Ser267Phe in SLC10A1.

OPEN PEER REVIEW

Revision (1)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | **Revision** (1) 8 Jun 2022

Dear Professor Alavian
Thank you for sending me the article entitled ": Biochemical and bioinformatic characterization of patients with
sodium taurocholate co-transporting polypeptide (NTCP) mutation" for review. This genomic study evaluated the effect of sodium taurocholate co-transporting polypeptide (NTCP) mutation on biochemical and bioinformatic characterization of patients. They divided the patients into 3 major groups and compared the mentioned variable among them. The study result showed that All
homozygous with p.Ser267Phe mutation are Hypercholanemia. I read the respect authors response to reviewers. I still have some concerns about the confounding factors (regarding the lipid profile and bile acid concentrations) that were not checked in this project. Were the subjects taking antihyperlipidemic agents excluded from the study? Were the insulin resistance status (HOMA-IR) evaluated in the participants? Another concern is the existence of disorders related to cholestasis (intra or extra hepatic types). Were the subjects with mentioned disorders excluded from the study?
I recommend the respect authors to clarify these shortages in the limitation section of their manuscript.

REFEREE: Associate Editor | **Revision** (1) 8 Jun 2022

Authors are still needed to further clarify the raised comments of the respected reviewer and consider the advised point in their revision to be submitted to the Journal.

Seyed Moayed Alavian: EIC | **Revision** (1) 9 Jun 2022

Dear Authors,

The peer-review team has asked for additional corrections before publication.

Regards,
EIC

OPEN PEER REVIEW

Revision (2)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

We appreciate the reviewer' precious comments and advice. Those comments are all valuable and very helpful for revising and improving our paper. We have revised the manuscript accordingly，and our point-by-point responses are presented below.

For Reviewer 1:

Dear Professor Alavian

Thank you for sending me the article entitled ": Biochemical and bioinformatic characterization of patients with sodium taurocholate co-transporting polypeptide (NTCP) mutation" for review. This genomic study evaluated the effect of sodium taurocholate co-transporting polypeptide (NTCP) mutation on biochemical and bioinformatic characterization of patients. They divided the patients into 3 major groups and compared the mentioned variable among them. The study result showed that All homozygous with p.Ser267Phe mutation are Hypercholanemia. I read the respect authors response to reviewers. I still have some concerns about the confounding factors (regarding the lipid profile and bile acid concentrations) that were not checked in this project. Were the subjects taking antihyperlipidemic agents excluded from the study? Were the insulin resistance status (HOMA-IR) evaluated in the participants? Another concern is the existence of disorders related to cholestasis (intra or extra hepatic types). Were the subjects with mentioned disorders excluded from the study? I recommend the respect authors to clarify these shortages in the limitation section of their manuscript.

R： Thank you for your precious comments and advice. We understand there are many factors that could affect serum BA level, which needs to be fully excluded before we diagnose *SLC10A1*^{S267F} mutation induced hypercholanemia. However, due to experimental conditions and equipment limitations, we only use medical history to excluded cholestasis or malabsorbtive disease or long-term medication history (such as anti-hyperlipidemic agents) in all recruited members. Because of the same reason, we did not evaluate insulin resistance status of recruited members. We have clarified these shortages in DISCUSSION section of our manuscript. In the future, we will attention to this topic and hope you may understand our difficulty at this stage. We appreciate your elaborate efforts in reviewing. Thank you very much!

OPEN PEER REVIEW

Revision (2)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

Seyed Moayed Alavian: EIC | **Revision** (2) 23 Jul 2022

Dear Authors,

I am delighted to inform that your revised manuscript is accepted for publication in Hepatitis Monthly.

Regards,
EIC