Peer Review File

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Reviewer A

Question: The image of optic nerves also shows pallor not just blurred sic margins. This is important to note and discuss.

Response: Thanks for your constructive suggestion. This photo was taken 1 month after visual loss so the pallor optic disc with blurred margin maybe caused by long term hypertension of intracranial pressure. And we have added these text in discussion.

Question: I am not impressed by the enhancement of hte ON on hte MRI .

Response: We truly appreciate your thoughtful advice; we have asked imaging specialist to review the MRI images and found that the gadolinium enhancement in left optic nerve in orbit segment was slightly probably because MRI images were scanned at 1 month after the optic neuritis onset and the inflammation in optic nerve had almost disappeared. Therefore, we had modified the text for P2: L71-73 "The orbit MRI scanning demonstrated thickening of the bilateral optic nerve in orbit, with enhancement by gadolinium in the left optic nerve (Figure 2)." as "The orbit MRI scanning demonstrated thickening of the left optic nerve in orbit, with slight enhancement remaining by gadolinium in orbit segment at 1 month after ON onset(Figure 2)." marked by blue font.

Most importantly there is need for IL1beta levels/activity since this is a new mutation.

Response: We truly appreciate your constructive advice. I'm always regret not to do protein function experiment about the genetic mutation. Your advice give me a easier way to confirm the genetic mutation. However, in 3 weeks we failed to contact the patient's guardian. Fortunately, we have diagnosed another patient, we'll assay serum IL1 level, thanks again.

Reviewer B

Overall, report introduced subject nicely. Review of literature gives detaile bit by bit. Report open symptoms and patient`s disease history. Treatment was mentioned as well as treatment output, but some detailes need to clarify and make consistent throughout manuscript, pointed out with detailes later. Report highlight different treatment options. Detailes of NLRP3 mutation and future perspective is mentioned.

At the beginning of the report there could be more information about NLRP3 gene mutation in general level related to the current disease and highligh more about what is already known about NLRP3 mutation related to the disease. Now, there is mentioned that mutation is first

reported in the present report. Later at the end of the report, there is given more detailes about previous findings of NLRP3 mutations. That was little bit confusing and it would be better open little bit more previous findings of NLRP3 gene mutations and output at the beginning of report. And then what new is reported in the present study and the meaning of that. Bring together spesific output of this research and tie to the previous knowledge.

Response: Thanks for your thoughtful suggestion. Most genetic mutation sites caused CINCA suited at NLRP3 and the c.913G>A (coding p. D305N) mutation site in NLRP3 gene was a novel and first reported not the whole gene NLRP3.I'm terribly sorry not descript them clearly and we have added the word site behind genetic mutation avoiding confusion. Thanks again.

TITLE
Abbreviation ON is little bit confusing in title and could be better to open. It is better open to the title and use abbreviation after it is opened first time in text. As it has done in line 22.
In addition, in the end of the title it is little bit confusing that there is "case report". Probably it does not need to write and could be informed by journal as usually. If not, probably it is better to inform that at the beginning. And after NLRP3, there could be ad word "gene".

Example:
"Case report: Bilateral optic neuritis caused by Chronic infantile neurological cutaneous and articular syndrome in a child with a novel (p. D305N) mutation in NLRP3 gene"

Response: Thanks for your thoughtful suggestion and we added "gene" behind the NLRP3 in title and deleted the abbreviation of ON. Thanks again.

Abstract

lines 18-20: "(CINCA) syndrome, is caused by the over-secretion of interleukin (IL)-1β due to a gain-of-function NLRP3 gene mutation"
It could be interested to see IL-1β levels in the particular case or at least it could be mentioned why it is not measured and is it even done routinely for patients. Or does it more belongs to the research of disease pathologic but not used as diagnostic.

Response: Thanks for so constructive and important suggestion. Your suggestion provides more perfect and feasible method instead of further protein function study. It's our negligence to do the work. In these days, we fail to call back this patient have IL-1 assay and we'll have IL-assay for CINCA patients in future. Thanks again.

lines 22-23
"Genetic testing revealed that the symptoms were caused by a novel gene mutation (c.913G>A, p. D305N) in NLRP3."

More information about gene tests and mutation could be nice to include and how that related to the CINCA disease onset.

It also would be interested to get more knowledge about background of the disease and NLRP3 mutations and what new the particular mutation exactly brings out. And what kind of spesific role of NLRP3 mutations are in the disease. Is it important for the disease onset or as secondary response? NLRP3 inflammasome activation and IL-1β secretion also belongs to the many other diseases onset and it woul be interested to highlight what kind of relationship NLRP3 has to the this particular disease.

Is there any method to detect NLRP3 gene mutation routinely example from fetus and start treatment earlier and slow down or prevent the disease onset?

Response: We appreciate your constructive suggestions. We added some information according to your suggestions "Genetic testing revealed that the symptoms were caused by a novel gene mutation site (c.913G>A, p. D305N) in conservative domain exon-3 of NLRP3 which is gain-function gene of CINCA." in line 25-27 and marked by blue font. However, due to the limitation of words in abstract, the details descriptions about CINCA and gene mutations see page 7 lines 238-252. For this case report, the new finding is the novel genetic mutations site (c.913G>A, p. D305N) in exon3 in NLRP3 gene which have not been reported before.

1. As for as the confirmed pathogenesis of CINCA caused by NLRP3 is still unclear now. The probably mechanism based on the recently studies is as following: the genetic site mutation in conservative domain exon3 in NLRP3 causes IL-1β over- secretion and then triggers the insult of autoinflammatory. Once triggered, NLRP3 interacts with other intracellular proteins and assemblies into a multiprotein complex called Inflammasome, a key player in IL-1β pathway activation. As aforementioned, once elicited, IL-1β pathway disruption occurring in cryopyrinopathies triggers a cascade of complex cellular events leading to aberrant homeostatic tissue responses and an extracellular oligomeric complex thus causing persistent inflammatory response (details see reference: Finetti M,Omenetti A,Federici S, et al. Chronic infantile neurological cutaneous and articular(CINCA) syndrome: a review. Orphanet J Rare Dis.2016; 11:167.)
2. As for "Is there any method to detect NLRP3 gene mutation routinely example from fetus and start treatment earlier and slow down or prevent the disease onset? "
   The CINCA diagnosis is confirmed, start IL-2 blockage treatment as possible as can immediately. Earlier treatment can prevent the further autoinflammatory damage. Some CINCA are caused by germline genetic mutation and some patients are caused by somatic genetic mutation. For CINCA family history positive mother, the fetus'gene test negative can't complete prevent the baby CINCA occurrence after birth. We think your advice is very important and have added this text in page 5 line 185.

line 32

There has done 3 years follow-up. It would be interested to see some table and values related to that from original level/measurements and the situation after 3 years follow. What exactly have measured.

Response: Thanks for your thoughtful advice. During 3-year follow-up, the autoinflammation

has not flared up and the system condition has been stable except for exophthalmos worsening and the visual acuity is stable. I'm afraid that there no valuable measurements to list in table, without quantitively observation indicators. The prognosis and follow-up data are recorded in Page5 line172-174 marked with blue fonts.

line 33
"The finding of novel genetic mutation (p. D305N) expanded genotype spectrum associated with CINCA."
This should be explained more and how NLRP3 mutations expanded and what there was already known about that. And what new information this particular report brings and what is the meaning of that. Also, something infromation about previous genetic mutations could add and is there some trend in those or are those just random point mutations. Is there similarities and differences.

Response: We appreciated your thoughtful advice. Due to the limitation of words in abstract, we have added these texts in discussion in page 7 line 240-254 and marked in blue fonts. Thanks again.

Keywords
"case report" maybe not need to use as keyword. It is more like scientific literature form and depends on what is the aim of publish. In addition, usually journal will inform in the detaile of that e.g article, review or case report.
- There could be added still few more keywords related to the particular study, disease, findings, treatment so. That would improve the visibility of research after publishing.
- keyword is coumpound word as "keyword" not "key word"

Response: Thanks for your constructive suggests and we have listed the keywords behind abstract in blue font as **"Keywords:** papilledema, chronic infantile neurological cutaneous and articular syndrome, novel genetic mutation site, IL-1 blockage agent.".

Introduction
-line 42, Abbreviation "CNS" need to open

Response: Thanks for your thoughtful advice. We have opened the abbreviation "CNS " for the first presentation in article.Thanks again.

line 45, "…ON case caused by CINCA with a novel a novel (p. D305N) mutation in NLRP3"
Two times a novel, and end of the NLRP3 could add word " gene"

Response: Thanks for your thoughtful advice. We have added "gene" after NLRP3 in line 45. Thanks again.

-lines 47-48, “The syndrome is caused by the over-secretion of interleukin (IL)-1β due to a gain-of-function NLRP3 gene mutation in the autosomal chromosome.””

Is there known more detailes about the effect of NLRP3 gene mutation related to the disease onset and how the impact of NLRP3 is detected? There could be more detailes or reviewed some research about that. Is there detected IL-1β levels? The levels of IL-1β at the beginning of the treatment and after follow-up period.

Response: Thanks for your constructive suggestions. We have added these content in page7 line245-249. As for the assay of IL-1 level in serum, we are sorry to fail to call back patient to do the job and we will do it in next patient.Thanks again.

It could also mention in introduction part that how NLRP3 mutation affect to the disease progression and is there measured also NLRP3 levels from patients. In addition, IL-1β release is two step process. First pro-IL-1β production is needed to produce through priming after which active NLRP3 inflammasome cleavage it into active secreted IL-1β form. Due to that, probably some other things also affect to the disease onset. Or does NLRP3 mutation cause priming of pro-IL-1β production? NLRP3 mutation impact probably could be interested to show somehow example by measuring NLRP3 levels from CSF samples.

Response: We really appreciated your comments and suggestion, and we have learned a lot from your comments. Due to clinical features of CINCA presented in patient and associated genetic tests positive, and excluding other possible inflammatory and infectious disease,we think the diagnose should be confirmed,therefore we don’t think of test NLRP3 level from CSF. Furthermore, based on previous studies, the type of genetic mutation had no related to the server degree of diseases. Thanks again.

Overall, it could be important review little bit more the meaning of NLRP3 mutation related to the CINCA. At the beginning of report, it is nicely reported that mutation is detected and IL-1β is main cause for CINCA but more detailes about mutation and meaning for CINCA and the IL-1β level detection could be informed. If that is known. It could be informative because NLRP3 and IL-1β are also part of many other diseases. It would be interesting to read more about pathological issues related to that. Or as an alternative more data e.g. NLRP3 and IL-1β levels detected from CSF from patient.

Response: We really appreciated your comments and suggestion. We try our best to persuade patient’parents come back to have IL-1 assay but we failed. In the future, we will do it in nest patient. Thanks again.

When reading further, in lines 131-132, it has told that NLRP3 is golden criteria for CINCA. Mutation is mentioned from the beginning but not exactly reviewed the meaning and output of the mutation and for which that claim golden criteria based so. Is it common criteria for the disease? And routinely used as diagnostics?

Response: We really appreciated your comments and suggestion. CINCA is very rare diseases, now we have not search a confirmed consensus criteria to diagnose CAPS or CINCA. Clincial features and laboratory tests are important to diagnose. In order to confirm the diagnosis the molecular analysis of NLRP3 gene is surely required. Due to the severe phenotype, usually de-novo mutations are detected. As already stated, up to 35–40% of patients with a clear CINCA/ NOMID phenotype turn out to be negative for germline mutations of NLRP3. Almost 70% of these patients are, instead, carriers of a somatic mosaicism, that can involve even a very low percentage of the cells of myeloid lineage. There is a general consensus among experts that the clinical picture of CINCA/ NOMID is sufficient to point out the diagnosis even in the absence of a positive genetic test (details see reference: Finetti M,Omenetti A,Federici S, et al. Chronic infantile neurological cutaneous and articular(CINCA) syndrome: a review. Orphanet J Rare Dis.2016; 11:167.).

case presentation
lines 63-64
Is it possible to measure and infrom the IL-1β and NLRP3 levels e.g. from CSF samples, if those are the main cause of the disease onset. It would be intrested to see the levels and how treatment affects to those or is it only clinical more interested to see the beneficial effects in the patient. That is of course the main aim, but measurements could be interested data related to the research of the CINCA and the treatments of the disease.

Response: We really appreciated your comments and suggestion. That's a good idea. However, for a confirmed diagnosis of CINCA patient by clinical presentation and genetic test, it is very to difficulty to persuade patient's guardian to consent to do invasive lumber puncture. I'm sorry for this. Thanks again.

Systemic medical history

- Overall, it would be nice to see more detailed the effect of mutation in NLRP3 gene. Now, it is showed nicely detailes of mutation and comapred to parents genomic. There is also reviewed that IL-1β is main cause of the disease onset. Still, it would be really interesting to show the exact effect of the mutation. Is it possible to measure secreted NLRP3 level e.g. from CSF samples from baseline and after treatment follow-up period?
In general, it has seen that activated NLRP3 is secreted out of the cells. Of course, that could be also more as local effect but probably could also be detected from CSF samples. Blood

sample levels could also be possible, if levels are not too low, but there could also be effect related to the other organ and maybe CSF sample is little bit more spesific for central nervous system. If compared to e.g parents or reference values of healty people that could probably give more detailes about situation and the effect of treatment. But of course, there could also be just local effect. However, it could also be little bit opened and reviewed if there is some literature about that.

Response: We really appreciated your comments and suggestion. We are all think it is a good ideal. But we are sorry to fail to assay serum IL-1 level and CSF NLRP3 level. We will do them in future, if it is possible. Regretfully, we failed to find the associated references about CINCA and IL-1 level in blood and CSF. If you have the associated references,would you like to let me know? Thanks sincerely.

Results
- lines 116-117
"Then she accepted steroid therapy combining with reduction of intracranial pressure with hyperosmotic solution."
There could be little bit more infromation about used treatments e.g. compound, concentration, dosing detailes.
And little bit more info about used treatments output and example exact valueas or how the output is detected. Does it based to the symptoms etc. Now, there is nicely reported out comes in patient`s physical response to treatment. But for repetition, research and later improvement of the treatment, more detailes could be intresting. Although, the aim is the better physical response in patient.

Response: We really appreciated your comments and suggestion.We have added "Then she accepted steroid therapy of methylprednisolone 20mg/kg·d taken intravenously for three days and prednisolone 2mg/kg·d taken orally and tapered by 5 mg per month, combining with reduction of intracranial pressure with 20% mannitol solution intravenously(5ml/kg, twice a day)."in page 4 line119-123.

-line 129
Abbreviation ANA and anti-ENA need to open when mentioned first time.
-There is nicely reported the symptoms of patients and criteria for CINCA. in lines 153-54 there is mentioned "Due to CINCA rarity to seen, few doctors could think of it. Even if considered of CINCA……"
Because of that there could be little bit more introduction related to criteria and symptoms of CINCA. There could even be table for the general criteria for CINCA compared to criteria founded from the particular patient with timeline. It would make the criteria for CINCA more visible if there is presented e.g general timeline for possible symptoms.

Response: We really appreciated your comments and suggestion.We have added the open name for ANA and anti-ENA in the first presentation. At the same time,we also added the timeline of symptoms image for CINCA patient in result part(Figure 3). Thanks again.

-It is little bit confusing at the beginning, if the patient got IL1 blocking agent or not. In lines 168-169 there is mentioned "Due to huge financial burden and unavailable of IL-1 blockage, she accepted steroid therapy of methylprednisolone 20mg/kg・d taken.." and in Table 1.there is more detailes about IL1 blocking treatment. It makes little bit confusing and probably there could be second table informed used treatment in the particular patient. Defenitely it is good point to mention IL1 blockin agent but also used tratment could be highlight more.
It could be informed more straigth from the beginning what treatment was used. And also estimate about real costs of both treatments because there was mentioned expensiveness of IL1 blocking treatment.

In lines 176-178 "order to reduce disabilities or organ function failures, the IL-1 blockage agents' treatment should be lifelong and should be given before organs sustain permanent injuries. Steroid therapy also was a sufficient treatment to prevent deterioration of diseases"

In lines above there is something reported used treatments compared to others. If there is proved that particular treatment is as good (or almost but not so expensive) as IL1 blocking it would indicate more clearly. Now, there is too much gap for readers`s interpretation when writers probably have best knowledge about the whole situation. That information could be beneficial for others too.

Later there was mentioned in lines 193-194 "Intervention adherence and tolerability. In china, the adherence of IL-1 blockage agents therapy was very poor because of huge financial burden, and steroid therapy was available."
If used therapy and response was good enough that would be mentioned and justify more. It would help others in same situation if there is informed options for IL1-blocking treatment. There also could be mentioned some supportive beneficial effects of used treatment or compared treatments.

Response: Thanks for your constructive suggestions. As for treatment for CINCA patients, in worldwide, especially for developed countries, the first line medicine is IL-1 blockage agents. However, as for this patient, at age of 3 years old, she was diagnosed as CINCA according to clinical symptoms and genetic test, and doctor persuaded her parents to have IL-1 blockage agents treatment. Patient' parents denied after consideration due to huge financial cost and life-long treatment. Therefore, doctor had no choice and only tried steroid treatment to prevent inflammation injury and successful controlled inflammation flare-up. But we have enough evidence to spread without big sample study. Avoiding the confusion, we revised this

part context in result and discussion. Thanks again.

line 183
Table texts are usually placed above the table

Response: Thanks for your careful advice, we have placed table text above the table. Thanks again.

lines 196-197
“For IL-1 blockage agents therapy, the allergic reaction and insufficiency after long-term usage were concerned.”
If in particular study there is not used IL1-blocking treatment, for avoiding confuse there should be references for the claim. Otherwise it seems that writers have noticed that themselves, which could also be mentioned if it is so.

Response: We really appreciated your comments and suggestion. It is really a concern of allergic reaction insufficiency. For allergic reaction of IL-1 blockage agents, we failed to find the associated report, but for insufficiency after long-term usage condition, doctors usually change other type of IL-1 blockage agent. We have added this text in manuscript.

Discussion
lines 202-204 “In this case report, the bilateral ON complicated to CINCA caused by a novel genetic mutation (D305N) in NLRP3 was first reported.”
Please clarify. If this particular mutation is new, it is good open little bit and review other known mutations. Probably the differences could also point out and if there is some differences for output of mutation in patients. Now, it was first little bit confusing if the NLRP3 mutation was detected first from all. Situation came little bit more opened later but it could open also from the beginning.
Now, all earlier genetic detailes comes at the end of the report and perspective little bit change when reading forward. Please clarify little bit the NLRP3 genetic affect throughout the report. And give some detailes how that spesific gene mutation was first and different than others and does all mutations affect same way to the onset of CINCA.

Response: We really appreciated your comments and suggestion.We have revised these text according to your advice and discussed it in paragraph 5 in discussion part.

Example above sentence is little bit inconsistent with lines lines 243-244 “Approximately 50–

69.2% of 244 CINCA/NOMID patients were detected mutations in the NLRP3 gene.(14,15)" Please clarify someway and consistent through report.

if this reported mutation first time and it is related to main cause for CINCA it needs more justification. Please clarify little bit the whole situation about NLRP3 mutations and the meaning of the particular finding to that.

Also please clarify and consistent above with lines 131-132, "Most important, genetic screen that found pathogenetic mutation in the NLRP3, was the golden criteria for diagnosis of CINCA."
Please clarify, is it common criteria for CINCA to detect NLRP3 mutation? Review little bit based to literature or modify sentence. Also highligh more how the particular mutation affect to the situation or is it only one mutation with others and affect same way.

lines 241-243, "CINCA/NOMID is a monogenic autoinflammatory disease with gain-of-function genetic mutation in the NLRP3 that encodes the NLRP3 protein, also called cryopyrin. These mutations caused IL-1 overexpression and triggered auto-inflammatory injuries."

If this is well known that NLRP3 is main reason for the disease it would be good to give some detailes about that and how it has indicated before. At least that can mention already in introduction. Also mention, if mutations can vary but output is same and some reference for that.

line 210 "..gene-function study was not performed".
Open little bit and is it planned to do future or not needed.

Response: We really appreciated your comments and suggestion. As for these questions we have made explanations and we don't want statement again. I think you are not a clinical doctor or you are doctor far away from clinical work or patients. In children' s hospital we often face with children patients who need life-time treatment with huge financial cost and have poor quality life. Once diagnosis are confirmed we will not have other tests especially for invasive examinations in order to save patients money and reduce physical pain. I am a doctor ,I love these children and love their parents.