Author’s response to reviews

Title: Isolated hypoaldosteronism as first sign of X-linked Adrenal Hypoplasia Congenita caused by a novel mutation in NR0B1/DAX-1 gene: a case report.

Authors:

Lorenzo Iughetti (lorenzo.iughetti@unimore.it)
Laura Lucaccioni (laura.lucaccioni@unimore.it)
Patrizia Bruzzi (bruzzi.patrizia@aou.it)
Silvia Ciancia (silvia.ciancia.18@gmail.com)
Elena Bigi (elena.bigi@gmail.com)
Smona Madeo (simonamadeo@hotmail.com)
Barbara Predieri (barbara.predieri@unimore.it)
Florence Roucher-Boulez (florence.roucher@chu-lyon.fr)

Version: 1 Date: 30 Apr 2019

Author’s response to reviews:

Dear Editor,

We would like to thank you for all the appreciated and useful comments we received.

Following our answers to the two reviewers. Changes in the manuscript are in bold red

To Reviewer n. 1

We would like to thank reviewer 1 for his kind assessment of our work. We edited the manuscript according to his comments as follow:

1) We have integrated the text as follow:

“Pathogenicity prediction was performed in silico using several programs: Align-GVGD, Polyphen-2 and SIFT and the variant was predicted to be most likely pathogenic using Align-
GVGD class, probably damaging, using Polyphen-2, deleterious using SIFT. The Grantham score that ranges from 0 to 215, was calculated to predict the effect of substitutions between amino acids based on chemical properties (i.e. polarity and molecular volume). Higher scores indicate greater differences between two amino acids and may indicate a stronger (negative) effect on protein structure and function. Physicochemical effect of this variation is important with a Grantham score of 76. Frequency databases (dbSNP, ESP, and gnomAD) were searched to determine if the variant had already been reported and it was not. It has not been found in 100 Caucasian healthy controls sequences. According to the ACMG/AMP standards Guidelines [Richards, et al. Standards and guidelines for the interpretation of sequence variants. 2015.] the variant is classified as pathogenic”. See page 6, lines 164-172.

For major clarity we have also made some minor changes:

Line 114: ‘gene’ shouldn’t be in italic.

Line 115: we changed the word “heterozygous” to “hemizygous’, because the mother has two X chromosomes

Line 125: CYP11B2 needed to be in italic

Line 132: NR0B1 needed to be in italic

Line 159 -163: ‘The deletions of two bases at the codons 848-849 and the insertions of two cytosines caused the substitution of a glycine with a proline (c.848_849delinsCC or p.(Gln283Pro)).’ It is not at the codon but at the base-pair (bp)” was changed into:

“An indel was identified with the deletion of two base-pair (bp) replaced by two cytosine in position 848_849. The sequence variants is designated according to the Human Genome Society recommendations (www.hgvs.org/rec.html) using the National Center for Biotechnology Information (NCBI) reference sequences NM_000475.4, NP_000466.2 built on the GRCh37/hg19 and is NM_000475.4:c.848_849delinsCC or p.(Gln283Pro)”.

2) In our patient glycemia was found always normal. We added the first measurement (see page 4, line 87)

3) Regarding mini puberty, we agree that levels of gonadotrophins and testosterone are at the highest of normal range for age. We have modified the text according to your suggestion and hope now it is clear (see page 4, line 109 and page 7, line 213).

4) Yes, adrenal autoantibodies were checked to exclude autoimmunity causes of adrenal insufficiency. We are aware that this is not the common age, but the family medical history was positive for autoimmune diseases and we ruled out every possible risk.
5) We added the paper suggested to Table 2 and References (see Reference n. 21).

To Reviewer n. 2

We would like to thank reviewer 2 for his kind assessment of our work. We edited the manuscript according to his comments as follow:

1) “The case was admitted to hospital for failure to thrive that is the symptom of hypocortisolism. Blood glucose level of the case was not given (hypoglycemia?).”

In a neonate (18 days old), failure to thrive may be linked to several different causes, from metabolic to endocrine or infectious diseases, from renal to cardiac misfunctions (As it is also pointed out by the paper of Bizzarri C et al, cited by the reviewer and now insert as part of our References list – see n. 22).

An immediate link between failure to thrive to hypocortisolism is not as simple as it could seem, especially if the baby is breastfed and there is no way to know the exact amount of milk assumed per day, and parents report that he is not eating as well as usual.

For brevity, we needed to point out only the main clinical characteristics of our patient, but the clinical history was not as clear as it is described. Moreover, glycemia was always normal even during the previous days when it was checked in another medical center. We added to the text the first glucose measurement done at admission to our department (see page 4, line 87).

2) “Normal plasma cortisol level doesn't rule out hypocortisolism whenever ACTH level was high. Mild pigmentation of the external genitalia was also the sign of hypocortisolism”.

As described through Twin A of the Al Amer et al paper, although first clinical manifestations appened during neonatal life, the complete adrenal insufficiency appeared only later in life. We should ask ourselves if this is the keystone of AHC itself. In fact, we agree that normal plasma cortisol level do not rule out hypocortisolism if ACTH is increased, but was ACTH level as high as expected at that age for complete adrenal insufficiency? What about the role of “pituitary and adrenal reserve” typical of the neonatal period?

We think that misdiagnosis may be more common at this age, and that clinicians need to be very careful approaching neonatal patients and their families. In fact, neonatal ACTH levels may be higher than values reported for older babies and, last but not least, in our case ACTH levels decreased once fludrocortisone was started and it raised again only months later.
Regarding the pigmentation of external genitalia, it was very mild, compatible with the normal pigmentation at this age, linked to increased sexual hormones and not to adrenal hypocortisolism (higher precursors of ACTH).

3) “The short Synacthen test was unnecessary in this case because of the high ACTH levels”.

We fully agree with this comment, but SST could have ruled out any doubt of misdiagnosis and it is considered the gold standard for hypocortisolism diagnosis from the whole scientific community.

4) “There are many similar cases of AHC that salt-wasting is the first presentation and diagnosed to have isolated primary hypoaldosteronism mistakenly (1,2).

In a study from Italy, authors investigated the data of newborns and infants that presented with hyponatremia in a single center during ten years. They found 51 infants, 19 of them diagnosed as CAH, and four of them diagnosed as AHC (2).”

We have now added the cases reported by these articles in Table 2, seen the outstanding value of these descriptions (see references list: 22 and 23).

5) “Table 2 is unnecessary. There are more than 200 NROB1/DAX-1 gene mutation cases in the literature”.

We prefer to maintain the table, seen that it could be an easy and useful instrument especially for young people approaching this rare condition for the first time. We added the cases of the articles suggested by the reviewer and hope that now may be considered more clear and suitable for publication.

6) “Discussion is successful and comprehensive to understanding the newborn mineralocorticoid regulation”. We thank the reviewer for this comment.

7) “This case, contains no new knowledge about AHC, except the NROB1/DAX-gene mutation was novel”.

We are aware that this clinical case may not present particular novelty, but the new mutation need to be known by the scientific community.
We hope that now the manuscript could be acceptable for publication in your journal.

Thanks for your attention.

Kind Regards,

Lorenzo Iughetti