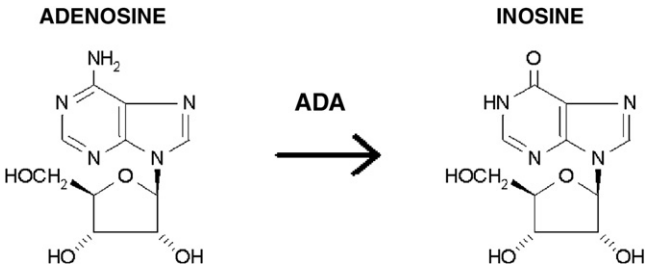

Fig. 1. Adenosine is deactivated by its rapid conversion to inosine through the hydrolysis of the purine amino group by ADA.

However, as pointed out by Valerio Napolioni, polymorphic variants have now been found with reduced rates of catalytic activity. In some circumstances, this is positively advantageous. Adenosine is released by cardiomyocytes in response to ischemia and is cardioprotective in this regard [13]. Genotypic variants resulting in reduced metabolism of, or increased receptor response to, adenosine result in a phenotypic group more likely to survive ischemic events [14,15]. The question to be investigated is this: do these same genotypes result in an IVF patient phenotype prone to the development of OHSS?

Ray Kruse Iles
Suzanne M. Docherty
Biomedical Sciences
School of Health & Social Science
Middlesex University, Hendon Campus
NW4 4BT The Burroughs, UK
E-mail address: r.iles@mdx.ac.uk

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How the "A" to "C" conversion may create a new splice acceptor site?

To the Editor:

We have read with great interest the article by Katsumata et al entitled "Novel intronic *CYP21A2* mutation in a Japanese patient with classic salt-wasting steroid 21-hydroxylase deficiency" [1].

The authors report a novel *CYP21A2* mutation (IVS-9A>C) at −9 position of intron 9 in a Japanese male patient suffering from a severe classic form of congenital adrenal hyperplasia.

Katsumata et al perform complex in vitro experiments showing that the transient expression of the *CYP21A2* IVS-9A>C mutation in COS-1 cells creates an aberrant splice acceptor site at −7 position of intron 9, inactivating the original slice acceptor site. The result is the complete deficiency of 21-hydroxylase activity and loss of immunoreactive CYP21A2 protein.

In line with the reported data, we now ask ourselves how the "A" to "C" conversion at −9 position of *CYP21A2* intron 9 may create a new splice acceptor site. Generally, a canonical acceptor splice site is "AG"; and only this consensus bases sequencing can be correctly recognized by splicing factors [2].

In the "Materials and methods" section, the authors declare the use of the *CYP21A2* nucleotides numbering according to Higashi et al [3]. This reference, like the one we used (M13936), reports at −9 position of *CYP21A2* intron 9 a "C" as wild-type base.

For this reason, the mutation described by Katsumata et al should be identified as IVS-9C>A and not as IVS-9A>C. In fact, the wild-type sequencing of the last nucleotides of intron 9 is acccgcccgc**ag** AT CGC TTC… (the original acceptor site splicing is in boldface); and the substitution of −9 "c" with an "a" can create a new "ag" acceptor splice site (underlined bases): acc<u>a</u>gcccgc**ag** AT CGC TTC…

Finally, we concord with the authors regarding the importance of reporting the discovery of novel *CYP21A2* mutations and performing their functional in vitro characterization. This can help to predict the correlation genotype/phenotype and may offer an appropriate genetic and prenatal counseling.

In addition, we want to underline the necessity to update the actual *CYP21A2* mutations databases [4] (unfortunately, the last update of the most accurate database, http://www.imm.Ki.se/CYPalleles/cyp21.htm, dates back to 2006.) and to correctly report the newly identified mutations, always indicating the sequencing reference number used (*CYP21A2* being a widely polymorphic gene, many errors can occur).

Paola Concolino
Cecilia Zuppi
Ettore Capoluongo
Laboratory of Molecular Biology
Institute of Biochemistry and Clinical Biochemistry
Catholic University, 00168 Rome, Italy
E-mail address: paolaconcolino78@libero.it

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Reply: IVS9-9C>A, not IVS9-9A>C, mutation in *CYP21A2* causes classic salt-wasting steroid 21-hydroxylase deficiency

To the Editor:

We thank Dr Concolino et al for their interest and comments regarding our recent publication on a *CYP21A2* mutation in *Metabolism* [1]. Just after online publication of this article, Dr Gonçalves (Centro de Genética Humana, Instituto Nacional de Saúde Dr Ricardo Jorge, Lisboa, Portugal) kindly pointed out our error in designating the mutation. As seen in Fig. 1A, the patient has a homozygous A at the IVS9-9 position [1]; thus, the mutation should have been designated IVS9-9C>A, not IVS9-9A>C. We already asked the Editor to add an "Erratum" section to correct the designation error, which has come out in the latest version of the article [1]. As discussed by Dr Concolino et al and demonstrated by our in vitro expression study [1], the IVS9-9C>A, not IVS9-9A>C, mutation creates a novel splice acceptor site and results in classic salt-wasting steroid 21-hydroxylase deficiency.

We concur with Dr Concolino et al in the necessity to update the *CYP21A2* databases and to discuss *CYP21A2* mutations always with the sequence references because the *CYP21A2* gene is highly polymorphic. We also emphasize the necessity to report new *CYP21A2* mutations with their functional consequences.

Noriyuki Katsumata
Takashi Shinagawa
Department of Molecular Endocrinology
National Research Institute
for Child Health and Development
Tokyo 157-8535, Japan
E-mail address: nkatsumata@nch.go.jp

Reiko Horikawa
Division of Endocrinology and Metabolism
National Center for Child Health and Development
Tokyo 157-8535, Japan

Kaori Fujikura
Sapporo City Institute of Public Health
Sapporo 003-8505, Japan

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