Physiol. Res. 59: 629-632, 2010

LETTER TO THE EDITOR

Comment on "Adiponectin and Resistin Gene Polymorphisms in Patients with Anorexia Nervosa and Obesity and Its Influence on Metabolic Phenotype"

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Received March 18, 2010

Comment on: KŘÍŽOVÁ J, DOLINKOVÁ M, LACINOVÁ Z, SULEK Š, DOLEŽALOVÁ R, HOUSOVÁ J, KRAJÍČKOVÁ J, HALUZÍKOVÁ D, BOŠANSKÁ L, PAPEŽOVÁ H, HALUZÍK M: Adiponectin and resistin gene polymorphisms in patients with anorexia nervosa and obesity and its influence on metabolic phenotype. *Physiol Res* **57**: 539-546, 2008.

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The paper "Adiponectin and Resistin Gene Polymorphisms in Patients with Anorexia Nervosa and Obesity and Its Influence on Metabolic Phenotype" by Křížová *et al*. (2008) presented original hypotheses and taking into account that presented research was performed on a population of the same ethnic origin, we decided to replicate the presented findings in our cohorts of subjects.

However, after a series of unsuccessful attempts to carry out the experiments described by the authors of the article, we discovered several major methodological problems within the paper.

First and foremost, the annotation of single nucleotide polymorphisms (SNPs) throughout the article is misleading and confusing. The authors claim to investigate the polymorphism –180C>G in the promoter region of the resistin gene (NM_020415) and they also report the –180C>G polymorphism not to have been investigated before; mentioning only one publication on –180C>G published so far (Smith *et al*. 2003). Unfortunately, it is highly likely that the authors did not take into account that the designation of –180C>G was actually originating from a different count method used by Smith *et al.* (2003), who employed a method of counting the positional assignment from the site of initiation of translation instead of the site of transcription start at nucleotide 3.168 within the resistin sequence (the accession number AF352730). When performing the proper positional assignment of the investigated polymorphism using the sets of primers reported in the paper, we came to conclusion that the authors were actually investigating SNP ID rs1862513, commonly recognized as –420C>G. However, this finding puts the whole work presented by the authors into a different light as plentiful of articles were published on –420C>G before 2008 (Engert *et al.* 2002, Ma *et al.* 2002, Cho *et al.* 2004, Osawa *et al.* 2004, Mattevi *et al*. 2004, Bouchard *et al.* 2004, Kunnari *et al.* 2005, Pistilli *et al.* 2007). Moreover, this polymorphism seems to be one of the most investigated SNPs within the resistin gene.

Furthermore, the authors also claim to investigate the +62G>A SNP in resistin gene. Based on the description provided by the authors, this polymorphism should be located in position +62, counted from the transcription or translation start site as it usual. However, the readers might get easily confused if they attempt to find such a polymorphism in +62 position of resistin gene sequence. We suggest that +62 G>A actually refers to +1326G>A, counted from the translation start site, (reference cluster ID rs3745368), which represents a SNP mentioned by Sentinelli *et al*.

(2002) in a paper focused on obesity and type 2 diabetes.

To avoid such confusions it would be highly advisable to quote the polymorphisms along with their valid SNP database reference cluster IDs, as it is a recognized a standard nowadays.

Last but not least, it is highly likely that the primer sequences for detection of polymorphisms in adiponectin gene (Tables 1 and 2) were not derived from the work by Xita *et al.* published in 2004, as the only paper of these authors published in 2004 was focused on a SNP in resistin, not adiponectin gene (Xita *et al*. 2004) and this paper contains no original data on adiponectin. In Table 3, the authors claim to use the set of primers for detection of +62G>A polymorphism in resistin gene, previously published by Tan *et al.* (2003). In fact, the paper by Tan *et al.* was investigating the resistin gene, unfortunately, instead of primers cited in Table 3 (forward primer 5‘-GCCGAGACCACATGTCACT-3‘, reverse primer 5‘-CCTCCGGGCCTACTAAAGAA-3‘), Tan *et al.* (2003) used a completely different set of primers (forward primer 5‘-AGAGTCCACGCTCCT GTGTT-3‘, reverse primer 5‘-CATCTCCAGGTTTAT TTCCAGC-3‘) for the detection of investigated polymorphism (Tan *et al.* 2003).

Finally, the sequence of the reverse primer reported in Table 4 to be used for detection of –180C>G (–420C>G) is misleading. Instead of reported sequence 5‘-AGATGCAGCAAAGCCAAAGT-3‘ (which actually does not exist within the resistin gene sequence), the correct sequence should be 5‘-GGGCTCAGCTAACCA AAT-3‘ as described by Smith *et al.* (2003).

To conclude, we presume that most of these confusions resulted from the improper work with information resources and therefore we would like to highlight the importance of verification of the previously published methods in order to avoid the persistent transmission of incorrect and misleading information confusing the future generations of investigators.

References

BOUCHARD L, WEISNAGEL SJ, ENGERT JC, HUDSON TJ, BOUCHARD C, VOHL MC, PERUSSE L: Human resistin gene polymorphism is associated with visceral obesity and fasting and oral glucose stimulated C-peptide in the Quebec Family Study. *J Endocrinol Invest* **27:** 1003-1009, 2004.

CHO YM, YOUN BS, CHUNG SS, KIM KW, LEE HK, YU KY, PARK HJ, SHIN HD, PARK KS: Common genetic polymorphisms in the promoter of resistin gene are major determinants of plasma resistin concentrations in humans. *Diabetologia* **47:** 559-565, 2004.

ENGERT JC, VOHL MC, WILLIAMS SM, LEPAGE P, LOREDO-OSTI JC, FAITH J, DORE C, RENAUD Y, BURTT NP, VILLENEUVE A, HIRSCHHORN JN, ALTSHULER D, GROOP LC, DESPRES JP, GAUDET D, HUDSON TJ: 5' flanking variants of resistin are associated with obesity. *Diabetes* **51:** 1629-1634, 2002.

KŘÍŽOVÁ J, DOLINKOVÁ M, LACINOVÁ Z, SULEK Š, DOLEŽALOVÁ R, HOUSOVÁ J, KRAJÍČKOVÁ J, HALUZÍKOVÁ D, BOŠANSKÁ L, PAPEŽOVÁ H, HALUZÍK M: Adiponectin and resistin gene polymorphisms in patients with anorexia nervosa and obesity and its influence on metabolic phenotype. *Physiol Res* **57:** 539-546, 2008.

KUNNARI A, UKKOLA O, KESANIEMI YA: Resistin polymorphisms are associated with cerebrovascular disease in Finnish Type 2 diabetic patients. *Diabet Med* **22:** 583-589, 2005.

MA X, WARRAM JH, TRISCHITTA V, DORIA A: Genetic variants at the resistin locus and risk of type 2 diabetes in Caucasians. *J Clin Endocrinol Metab* **87:** 4407-4410, 2002.

MATTEVI VS, ZEMBRZUSKI VM, HUTZ MH: A resistin gene polymorphism is associated with body mass index in women. *Hum Genet* **115:** 208-212, 2004.

OSAWA H, YAMADA K, ONUMA H, MURAKAMI A, OCHI M, KAWATA H, NISHIMIYA T, NIIYA T, SHIMIZU I, NISHIDA W, HASHIRAMOTO M, KANATSUKA A, FUJII Y, OHASHI J, MAKINO H: The G/G genotype of a resistin single-nucleotide polymorphism at -420 increases type 2 diabetes mellitus susceptibility by inducing promoter activity through specific binding of Sp1/3. *Am J Hum Genet* **75:** 678-686, 2004.

PISTILLI EE, GORDISH-DRESSMAN H, SEIP RL, DEVANEY JM, THOMPSON PD, PRICE TB, ANGELOPOULOS TJ, CLARKSON PM, MOYNA NM, PESCATELLO LS, VISICH PS, ZOELLER RF, HOFFMAN EP, GORDON PM: Resistin polymorphisms are associated with muscle, bone, and fat phenotypes in white men and women. *Obesity (Silver Spring)* **15:** 392-402, 2007.

SENTINELLI F, ROMEO S, ARCA M, FILIPPI E, LEONETTI F, BANCHIERI M, DI MARIO U, BARONI MG: Human resistin gene, obesity, and type 2 diabetes: mutation analysis and population study. *Diabetes* **51:** 860-862, 2002.

SMITH SR, BAI F, CHARBONNEAU C, JANDEROVA L, ARGYROPOULOS G: A promoter genotype and oxidative stress potentially link resistin to human insulin resistance. *Diabetes* **52:** 1611-1618, 2003.

TAN MS, CHANG SY, CHANG DM, TSAI JC, LEE YJ: Association of resistin gene 3'-untranslated region +62G-->A polymorphism with type 2 diabetes and hypertension in a Chinese population. *J Clin Endocrinol Metab* **88:** 1258-1263, 2003.

XITA N, GEORGIOU I, TSATSOULIS A, KOURTIS A, KUKUVITIS A, PANIDIS D: A polymorphism in the resistin gene promoter is associated with body mass index in women with polycystic ovary syndrome. *Fertil Steril* **82:** 1466-1467, 2004.

Answer to Hlavna *et al.*

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Received May 17, 2010

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We thank Hlavna and colleagues for their comments to our paper. We are sorry that the authors were confused by our methodological part and we would have been certainly more than happy to provide any additional explanations to our methodology at any time if we were asked to which nevertheless was not case.

The authors criticize numerous part of our methodology. Unfortunately, they do not provide any concrete data from their laboratory to support their conclusions. Furthermore, although the authors certainly have some publication record to support their expertise in testing adiponectin gene polymorphism in patients with obesity or atherosclerosis they to our best knowledge did not publish any papers either on resistin gene polymorphisms or in a cohort of patients with anorexia nervosa which one would have expected in the light of strong conclusions they made with respect to our paper and its scientific significance.

In our paper, we used the term for resistin polymorphism –180C>G according to the study by Smith *et al*. (2003) this polymorphism can also be termed –420C>G. We are sorry to disagree with Hlavna and his colleagues that scientific value of our findings is low in the view of this clarification. To our best knowledge there are no published papers studying this polymorphism in patients with anorexia nervosa to date and comparing its frequency in these patients with patients with obesity as

we did in our work.

We indeed investigated the +62G>A SNP in resistin gene and this position actually refers to +1085G>A and not to +1326G>A as Hlavna and colleagues are suggesting. The set of used primers and entire methodology are correctly described in our work in Table 3. On the other hand we acknowledge some confusion in our Methods description since the set of primers was designed in our laboratory and the method was also optimized in our laboratory and this was not clearly enough described in our paper.

The primer sequences for detection of polymorphisms in adiponectin gene (Tables 1 and 2) were not derived from the work by Xita *et al.* (2004), but from the work by the same authors published a year later (Xita *et al.* 2005) as Hlavna and colleagues correctly pointed out and we appreciate this correction. The sequence of reverse primer for determination of SNP –180C>G in resistin gene in Table 4 of our paper was in fact the primer sequence for detection of SNP +276 G>T in adiponectin gene. We apologize for this mistake that was a result of improper copying of primer sequences within the text. Determination of SNP –180C>G in resistin was designed as it was published in the work by Smith *et al*. (2003).

We would like to stress that our study was meant as a pilot one and all of the results were certainly affected by the relatively low number of subjects in our cohort for the standards of gene polymorphism studies. However, we have clearly identified these limitations in the last paragraph of the Discussion of our paper. It should also be mentioned that while there is a number of papers published on this topic in patients with obesity and/or type 2 diabetes mellitus, there is a lack of information on this topic in patients with anorexia nervosa. With respect to this issue, we did not find any citation in Hlavna´s letter related to studies in patients with anorexia nervosa that would support their conclusion about low originality of our results.

We certainly appreciate if any of our papers is cited and stimulates a fruitful scientific discussion. We also acknowledge above described methodological incorrectness on our site and we again thank Hlavna and colleagues for identifying it. On the other hand, we do have to insist that any criticism of previously published data has to be based either on a strong previous publication record or on the concrete data of the opponent (or in the very least of others) showing clearly that the criticized paper was wrong or not original enough. Only if these conditions are met and the authors of original paper have access to complete results, methodology, groups of subjects, their characterization and all other standard features of their opponents´ work the fair final conclusion can be drawn. We do agree with Hlavna and colleagues that such properly derived conclusion might be very useful to avoid the persistent transmission of incorrect and misleading information confusing the future generations of investigators.

Acknowledgement

Original authors´ study discussed in this paper was supported by grant of Grant Agency of the Czech Republic 301/04/P167 and MZOVFN2005.

References

SMITH SR, BAI F, CHARBONNEAU C, JANDEROVA L, ARGYROPOULOS G: A promoter genotype and oxidative stress potentially link resistin to human insulin resistance. *Diabetes* **52:** 1611-1618, 2003.

XITA N, GEORGIOU I, TSATSOULIS A, KOURTIS A, KUKUVITIS A, PANIDIS D: A polymorphism in the resistin gene promoter is associated with body mass index in women with polycystic ovary syndrome. *Fertil Steril* **82:** 1466-1467, 2004.

XITA N, GEORGIOU I, CHATZIKYRIAKIDOU A, VOUNATSOU M, PAPASSOTIRIOU GP, PAPASSOTIRIOU I, TSATSOULIS A: Effect of adiponectin gene polymorphisms on circulating adiponectin and insulin resistance indexes in women with polycystic ovary syndrome. *Clin Chem* **51**: 416-423, 2005.