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RESEARCH LETTER

Gain-of-function SNPs in *NLRP3* and *IL1B* genes confer protection against obesity and T2D: undiscovered role of inflammasome genetics in metabolic homeostasis?

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Introduction

Inflammation represents a cause, but also a consequence of obesity, contributing to disrupt metabolic homeostasis. Previously associated to high fat diets and physical inactivity, the contribution of gut microbiota and genetic inheritance are also being evaluated as risk factors for obesity. [1]

Increasing, and sometimes contrasting, findings have revealed the involvement of inflammasome in the development of obesity and obesity-associated diseases in experimental models. Inflammasome is a cytoplasmic complex responsible for the activation of caspase-1 and the consequent production of the biologic active form of interleukin (IL)-1ß and IL-18. Several intracellular innate receptors (i.e., NLR Family Pyrin Domain Containing 1/ NLRP1, NLRP3, NLRP6, Absent in Melanoma 2/AIM2) sense pathogen-associated and danger-associated molecular patterns (DAMPs), respectively, and became able to mount an inflammasome. These molecules are expressed in leucocytes, as well as in non-immune cells, such as adipose tissue and epithelium. In intestinal epithelial cells (IEC), it has been demonstrated that specific innate receptors act not only fighting against pathogens, but also regulating gut microbiota [1].

Moreover, once obesity has been established, several obesity-related DAMPs (i.e., high levels of glucose, fatty acids, uric acid), oxidative and/or endoplasmic reticulum (ER) stress are responsible for inflammasome-dependent secretion of IL-1ß and IL-18 from both leucocytes and adipocytes, leading to a dangerous pro-inflammatory loop [2].

In humans, it is possible to observe an increasing of both inflammasome cytokines in serum from obese individuals [3, 4]. However, it is difficult to understand whether the activation of inflammasome has been the trigger or, eventually, an important contributing factor for the development of obesity, or whether it "simply" amplifies the metabolic stress induced inflammation.

The aim of this study was to elucidate the contribution of inflammasome genetics in the development of obesity and obesity-associated morbidities.

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Material and methods

Patients

Two hundred and eighty eight unrelated obese patients (male/female: 22/266) and 96 non-obese individuals (male/ female: 19/77) were recruited from the Department of Obesity, Centre of Medical Specialties of Minas Gerais (Belo Horizonte, Southeast of Brazil) in compliance with the Institutional Review Board of the "Santa Casa de Belo

Horizonte" hospital. Body mass index (BMI) was used to define obesity: subjects with BMI ≥ 30 kg/m² were classified as obese, BMI of 25–29.9 kg/m² as over-weight and BMI of 18.5–24.9 kg/m² as normal weight [5]. Due to the limited number of over-weight individuals in our cohort ($n = 3$), and considering their border-line BMI values (25.03; 25.33 and 27.10, respectively), they were grouped with the normal-weight group. Median BMI of obese group is 45.17 Kg/m² (min: 30.41-max: 82.83), and of non-obese group is 22.68 Kg/m² (min: 17.90-max: 27.10).

To determine metabolic risk factors in obese subjects, we followed the diagnostic criteria for metabolic syndrome defined in accordance with the American Heart Association/ National Heart, Lung, and Blood Institute Scientific Statement [6], which included systemic arterial hypertension (SAH) (systolic blood pressure >140 mmHg, and/or diastolic blood pressure >90 mmHg, and/or drug treatment for elevated blood pressure) and type-2 diabetes (T2D) (elevated fasting glucose ≥ 100 mg/dL or drug treatment for elevated glucose). One hundred and six out of 288 obese, and 35 out of 96 non-obese individuals have SAH. Seventy three (male/female: 6/67) out of 288 obese, and 6 (male/ female: 1/5) out of 96 non-obese individuals have T2D. For 18 obese individuals (male/female: 3/15) we did not have clinical data about T2D. Sixty seven (male/female: 6/61) out of 288 obese, and 5 (male/female: 1/4) out of 96 non-obese individuals have both SAH and T2D.

SNPs genotyping

Five SNPs were selected based on minor allele frequency, functional effect and/or previously reported association with human disorders: NLRP1 rs12150220 (L155H), rs11651270 (M1184V); NLRP3 rs10754558 (3'UTR); IL1B rs16944 (promoter); IL18 rs1834481 (non-coding transcript exon variant/intron) [7]. SNPs genotyping was performed using allele-specific Taqman assays (Applied Biosystems) and qPCR using the QuantStudio3 Real-Time PCR platform (Applied Biosystems).

Data analyses

The effect of SNPs on clinical and biochemical variables was analysed by multivariate association based on general linear model adjusted for confounders variables (age, sex, ethnicity) using R-project package "SNP-assoc" version 1.9–2. Analyses were performed in the codominant, dominant, recessive and overdominant models for all SNPs. The Haploview software was used to investigate the linkage disequilibrium (LD) and to derive the haplotypes. A significant threshold of $p = 0.01$ has been assumed after Bonferroni correction for multiple SNPs analysis ($p = 0.05/$ n; $n = 5$ SNPs).

Results and discussion

In the present study, we evaluated the genetic contribution of inflammasome-encoding genes NLRP1, NLRP3, IL1B, IL18 in the development of obesity and obesity-associated comorbidities, precisely SAH and T2D. All the polymorphisms resulted in Hardy–Weinberg equilibrium. No LD was found for NLRP1 SNPs.

Nor NLRP1 nor IL18, which have been recently showed to play a key role in obesity in animal model [8, 9], were associated to obesity or related diseases, at least in our cohort (Supplementary File 1).

On the other hand, NLRP3 gain-of-function variant rs10754558 resulted significantly associated to obesity (p_{adj} $= 0.009$) (Table 1). NLRP3 rs10754558 G minor allele resulted more frequent in non-obese than in obese individuals (C/G+G/G: 0.68 vs. 0.55; OR = 0.46, according to a dominant model of inheritance) (Table 1), suggesting a protective role of this variant against obesity development.

When BMI, as a continuous variable, was considered, NLRP3 rs10754558 continued to be associated with obesity. Individuals carrying NLRP3 rs10754558 C/G genotype presented lower BMI values compared to homozygotes (C/ G: 38.87 ± 1.01 vs. C/C+G/G: 42.40 ± 0.94; $p_{adj} = 0.015$, according to an over-dominant model of inheritance) (Table 1).

The protective role of this NLRP3 rs10754558 variant in obesity is quite unexpected. rs10754558 SNP is localized in 3'UTR region of NLRP3 and determines an increased NLRP3 mRNA stability possibly affecting the micro RNA-223 binding site, cooperating to increase transduction rate of NLRP3 mRNA [10]. It increases the secretion of IL-1ß and IL-18 by human peripheral blood monocytes or monocyte-derived macrophages stimulated with bacterial lipopolysaccharides (yet unpublished data).

Activation of NLRP3 inflammasome and the resulting production of pro-inflammatory cytokines have been showed to play a major role in adipogenesis and in proinflammatory transformation of adipose tissue in chronical overfeeding mice [11].

However, we must consider the physiological role of inflammasome [12] in maintaining body homeostasis. A basal activation of inflammasomes is required to control microbiota, IEC proliferation and tissue repair through the production of IL-18 [13]. IL-18 appeared to exert a paradoxical effect in obesity: obese individuals present increased serum level of the cytokine [3, 14], nonetheless it has been recently demonstrated that IL-18 represents a major player against obesity [8, 9]. Vijay-Kumar and Gewirtz discussed the role of NLRP3 in obesity, emphasizing that the deficiency of NLRP3 leads to an altered microbiota predisposing to the development of obesity and obesity-associated morbidities in mice [15]. Altogether

Table 1 Detailed results for association of *NLRP3* rs10754558 and *IL1B* rs16944 with obesity and type-2 diabetes

Rs10754558	Obese ($n = 288$)	Non-obese ($n = 96$)	p_{adj}	OR_{adj} (95% CI)
C/C	0.45	0.32	0.009 (dom)	Ref
C/G+G/G	0.55	0.68		0.46 (0.25–0.84)
Rs10754558	Individuals (Frequency)	BMI (Mean ± SE)	p_{adj}	Dif (low/up)
C/C+G/G	0.63	42.40 ± 0.94	0.007 (over)	−3.69
C/G	0.37	38.87 ± 1.01		(−6.35/−1.03)
Rs16944	No T2D ($n = 215$)	T2D ($n = 73$)	p_{adj}	OR_{adj} (95% CI)
G/G	0.33	0.49	0.024 (dom)	Ref
G/A+A/A	0.67	0.51		0.49 (0.32–0.92)
Rs16944	No T2D female ($n = 184$)	T2D female ($n = 67$)	p_{adj}	OR_{adj} (95% CI)
G/G	0.37	0.59	0.001 (dom)	Ref
G/A+A/A	0.63	0.41		0.41 (0.24–0.71)
Rs16944	No T2D >40 years ($n = 90$)	T2D >40 years ($n = 57$)	p_{adj}	OR_{adj} (95% CI)
G/G	0.60	0.39	0.009 (dom)	Ref
G/A+A/A	0.40	0.61		0.44 (0.23–0.83)

NLRP3 rs10754558 and *IL1B* rs16944 association with obesity and type 2 diabetes (T2D) respectively, was analysed by general linear model (GLM). Genotypes distribution in obese ($n = 288$; BMI ≥ 30) and non-obese ($n = 96$; BMI < 30) individuals, p-value adjusted for sex, age, race (p_{adj}), Odds Ratio and respective 95% confidence intervals are reported. The analysis of association of rs16944 with T2D is made only in obese subjects. Model of inheritance (according with Akaike information criterion index) is indicated within brackets (*dom* dominant, *over* overdominant)

these findings help us to explain our quite unexpected result.

Finally, when we have considered obesity-related comorbidities, namely SAH or T2D, we observed no association among SNPs and SAH (Supplementary File 1), but a significant association between *IL1B* rs16944 and T2D (Table 1).

IL1B rs16944 minor A allele resulted less frequent in T2D ($n = 73$; male/female: 6/67) than in non-T2D ($n = 215$; male/female: 31/184) patients (G/A+A/A: 0.51 vs. 0.67; $p_{adj} = 0.008$; OR = 0.49), especially in women ($p_{adj} = 0.001$; OR = 0.41), or in older individuals (>40 years; T2D: 57 out of 73; non-T2D: 90 out of 215) ($p_{adj} = 0.009$; OR = 0.44).

Once again, a gain-of-function of inflammasome was associated with protection against the development of diabetes. In mice, IL-1β has been pointed out as one of the factors involved in the translation of obesity-associated inflammation into insulin resistance (as largely reviewed in ref. [16]).

All this considered, we hypothesized two scenarios, one in which the genetic background shapes a predisposing (loss of NLRP3 or NLRP6 in mice [17]) or protective (gain-of-function polymorphisms in inflammasome genes) backbone against obesity; and another, in which, in a gain-of-weight environment, the NLRP3-inflammasome senses cell stress agents and heavily contributes to adipose tissue inflammation [11] and consequently to the pathogenesis of obesity and metabolic disorders.

Compliance with ethical standards

References

1. L.P. Zambetti, A. Mortellaro, NLRPs, microbiota, and gut homeostasis: unravelling the connection. J. Pathol. **233**(4), 321–330 (2014)
2. M. Haneklaus, L.A. O'Neill, NLRP3 at the interface of metabolism and inflammation. Immunol. Rev. **265**(1), 53–62 (2015)
3. K. Esposito, A. Pontillo, M. Ciotola, C. Di Palo, E. Grella, G. Nicoletti, D. Giugliano, Weight loss reduces interleukin-18 levels in obese women. J. Clin. Endocrinol. Metab. **87**(8), 3864–3866 (2002)

4. A. Satpathy, S. Ravindra, S. Thakur, S. Kulkarni, A. Porwal, S. Panda, Serum interleukin-1β in subjects with abdominal obesity and periodontitis. Obes. Res. Clin. Pract **9**(5), 513–521 (2015)

5. K.M. Flegal, B.K. Kit, H. Orpana, B.I. Graubard, Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis. JAMA **309**(1), 71–82 (2013)

6. S.M. Grundy, J.I. Cleeman, S.R. Daniels, K.A. Donato, R.H. Eckel, B.A. Franklin, D.J. Gordon, R.M. Krauss, P.J. Savage, S.C. Smith Jr, J.A. Spertus, F. Costa; American Heart A, National Heart L, Blood I, Diagnosis and management of the metabolic syndrome: an American heart association/national heart, lung, and blood institute scientific statement. Circulation **112**(17), 2735–2752 (2005)

7. Y. Zhong, A. Kinio, M. Saleh, Functions of NOD-like receptors in human diseases. Front. Immunol. **4**, 333 (2013)

8. A.J. Murphy, M.J. Kraakman, H.L. Kammoun, D. Dragoljevic, M.K. Lee, K.E. Lawlor, J.M. Wentworth, A. Vasanthakumar, M. Gerlic, L.W. Whitehead, L. DiRago, L. Cengia, R.M. Lane, D. Metcalf, J.E. Vince, L.C. Harrison, A. Kallies, B.T. Kile, B. A. Croker, M.A. Febbraio, S.L. Masters, IL-18 production from the NLRP1 inflammasome prevents obesity and metabolic syndrome. Cell. Metab. **23**(1), 155–164 (2016)

9. M.G. Netea, L.A. Joosten, The NLRP1-IL18 connection: a stab in the back of obesity-induced inflammation. Cell. Metab. **23**(1), 6–7 (2016)

10. Y. Hitomi, M. Ebisawa, M. Tomikawa, T. Imai, T. Komata, T. Hirota, M. Harada, M. Sakashita, Y. Suzuki, N. Shimojo, Y. Kohno, K. Fujita, A. Miyatake, S. Doi, T. Enomoto, M. Taniguchi, N. Higashi, Y. Nakamura, M. Tamari, Associations of functional NLRP3 polymorphisms with susceptibility to food-induced anaphylaxis and aspirin-induced asthma. J. Allergy. Clin. Immunol. **124**(4), 779–785 (2009). e6

11. R. Stienstra, J.A. van Diepen, C.J. Tack, M.H. Zaki, F.L. van de Veerdonk, D. Perera, G.A. Neale, G.J. Hooiveld, A. Hijmans, I. Vroegrijk, S. van den Berg, J. Romijn, P.C. Rensen, L.A. Joosten, M.G. Netea, T.D. Kanneganti, Inflammasome is a central player in the induction of obesity and insulin resistance. Proc. Natl Acad. Sci. USA **108**(37), 15324–15329 (2011)

12. E. Elinav, J. Henao-Mejia, R.A. Flavell, Integrative inflammasome activity in the regulation of intestinal mucosal immune responses. Mucosal Immunol. **6**(1), 4–13 (2013)

13. M. Levy, E. Blacher, Elinav E microbiome, metabolites and host immunity. Curr. Opin. Microbiol. **35**, 8–15 (2016)

14. J. Hung, B.M. McQuillan, C.M. Chapman, P.L. Thompson, J.P. Beilby, Elevated interleukin-18 levels are associated with the metabolic syndrome independent of obesity and insulin resistance. Arterioscler. Thromb. Vasc. Biol. **25**(6), 1268–1273 (2005)

15. M. Vijay-Kumar, A.T. Gewirtz, Is predisposition to NAFLD and obesity communicable? Cell. Metab. **15**(4), 419–420 (2012)

16. C. Bing, Is interleukin-1β a culprit in macrophage-adipocyte crosstalk in obesity? Adipocyte **4**(2), 149–152 (2015)

17. J.1 Henao-Mejia, E. Elinav, C. Jin, L. Hao, W.Z. Mehal, T. Strowig, C.A. Thaiss, A.L. Kau, S.C. Eisenbarth, M.J. Jurczak, J.P. Camporez, G.I. Shulman, J.I. Gordon, H.M. Hoffman, R.A. Flavell, Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature **482**(7384), 179–185 (2012)