Molecular Biology Reports

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--Manuscript Draft--

Manuscript Number:	
Full Title:	Association of resistin polymorphisms with resistin levels and lipid profile in children.
Article Type:	Manuscript
Keywords:	children; lipid levels; resistin levels; resistin polymorphisms.
Corresponding Author:	Carmen Garces, Ph.D. IIS-Fundacion Jimenez Diaz Madrid, SPAIN
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	IIS-Fundacion Jimenez Diaz
Corresponding Author's Secondary Institution:	
First Author:	Lorena Ortega
First Author Secondary Information:	
Order of Authors:	Lorena Ortega
	Pilar Navarro
	Pia Riestra
	Teresa Gavela-Pérez
	Leandro Soriano-Guillén
	Carmen Garces, Ph.D.
Order of Authors Secondary Information:	
Suggested Reviewers:	Jose C Florez Harvard Medical School jcflorez@partners.org
	Hiroshi Onuma Ehime University Graduate School of Medicine onuma@m.ehime-u.ac.jp
	Haruhiko Osawa Ehime University School of Medicine harosawa@m.ehime-u.ac.jp
	Olavi Ukkola Oulu University Hospital olavi.ukkola@oulu.fi
	Cia-Hin Lau University of Malaya lauciahin_4275@yahoo.com
	Mitsuhiro Yokota Aichi-Gakuin University myokota@dpc.aichi-gakuin.ac.jp

Association of resistin polymorphisms with resistin levels and lipid profile in children‡

Lorena Ortega[1], Pilar Navarro[1], Pía Riestra[1], Gavela-Pérez Teresa[2], Leandro Soriano-Guillén[2], Carmen Garcés[1]

[1]Lipid Research Laboratory, IIS-Fundación Jiménez Díaz, Madrid, Spain.

[2]Department of Pediatrics, IIS-Fundación Jiménez Díaz, Madrid, Spain.

‡ Dedicated to the late Prof. Manuel de Oya, as the warmest homage to his memory.

Corresponding author:

Carmen Garcés

Lipid Research Laboratory

IIS-Fundación Jiménez Díaz

Avda. Reyes Católicos, 2. 28040 Madrid, Spain

Telephone/fax: +34-91-5432880

cgarces@fjd.es

Abstract

Background: Previous research has found a correlation between resistin and lipid level variations. Polymorphisms in the resistin gene (*RETN*) could be involved in this relationship, but the results of the different studies are contradictory. The aim of this study was to examine the association between resistin and lipid levels and to determine whether resistin polymorphisms are associated with resistin levels and lipid profile in prepubertal children and adolescents.

Methods: The single nucleotide polymorphisms (SNPs) rs1862513 and rs10401670 were analyzed in 442 randomly selected 6- to 8-year-old children and 827 children aged 12 to 16 years. Anthropometric data were recorded. Lipid profile was determined using standard methods. Serum resistin levels were measured using a multiplexed bead immunoassay. Resistin polymorphisms were determined by TaqMan® allelic discrimination assays.

Results: A relationship was found between serum levels of resistin and the SNP rs10401670 in 6- to 8-year-old boys. SNP rs10401670 was also related to TC and LDL-cholesterol in 12- to 16-year-old boys and to HDL-C in 12- to 16-year-old girls. SNP rs1862513 was not related to any of the studied variables. Serum resistin levels were significantly and negatively associated with ApoAI levels in 12- to 16-year-old girls.

Conclusions: A SNP in the 3´ UTR region of *RETN* (rs10401670) is associated with resistin levels and lipid profile in children, showing different associations depending on age and gender.

Keywords: children; lipid levels; resistin levels; resistin polymorphisms.

Introduction

Resistin is an adipokine secreted by adipocytes and by macrophages in adipose tissue and the liver [1]. It has been linked to obesity and obesity-associated alterations, although its precise role in metabolic disorders is under debate [2, 3]. Resistin levels have been associated with variations in lipid levels in several adult populations [4-11]. A link between this cytokine and obesity has also been reported in children [12-14], but few studies have investigated its association with lipid profile in children [15, 16].

The gene encoding resistin (*RETN*) is located on chromosome 19p13. A high heritability of plasma resistin levels has been suggested [17]. Several single-nucleotide polymorphisms (SNPs) described in the resistin gene have been associated with resistin levels [18-23]. The association of these SNPs with anthropometric variables and obesity-related alterations, including lipid profile variations, has also been studied, producing inconsistent results; for example, SNP rs1862513, one of the most extensively studied SNPs, has been related with obesity [24], body mass index [25, 26], and lipid level variations [22, 27], but studies in other populations have failed to find its association with anthropometric variables [22, 24, 28] or lipid profile [17, 19, 25]. The association of the SNP rs1862513 with obesity has also been the subject of studies in children [29], but to our knowledge its association with lipid levels has not been investigated in this age group.

In our study we have genotyped two SNPs in *RETN* (rs1862513 and rs10401670) that have previously been related to resistin levels in other populations, and we have analyzed their relationship with resistin levels, anthropometric measurements, and lipid profile in population-based cohorts of healthy pre-pubertal children and adolescents between the ages of 12 and 16 years.

Materials and Methods

Subjects: The study population included 2 population-based samples comprising 442 (48% boys) 6- to 8-year-olds and 827 (47% boys) 12- to 16-year-olds. The children were participants in a cross-sectional study conducted to analyze cardiovascular risk factors in Spanish schoolchildren. Parents were required to provide written consent for their children to participate in the study. All children reported by their parents to be suffering from chronic diseases were excluded. The study protocol complied with the Helsinki Declaration guidelines and Spanish legal provisions governing clinical research on humans, and was approved by the Clinical Research Ethics Committee of the Fundación Jiménez Díaz in Madrid.

Anthropometric variables: Measurements were taken with children wearing light clothing and barefoot. Weight was determined to the nearest 0.1 kg using a standardized digital scale and height was measured to the nearest 0.1 cm using a portable stadiometer. Body mass index (BMI; weight in kilograms/height in meters squared) was calculated and z-score BMI was determined according to the reference population [30]. Children were classified as normal-weight or overweight according to the age- and sex-specific cut-off points for BMI proposed for children by Cole et al. in a synthesis of international studies [31].

Biochemical data: Fasting (12h) venous blood samples were obtained by venipuncture and collected in Vacutainer tubes. Serum resistin levels were quantified using multiplex assay kits that utilize fluorescent microbead technology. A customized panel from BioRad (Bio-Plex Pro™ Human Diabetes Standard 10-Plex; Bio-Rad, Hercules, CA, USA) was used in the Luminex 200 System platform (Luminex Corporation, Invitrogen; Caramillo, CA, USA). Assay working ranges were 2.3-4739 pg/mL.

DNA extraction and polymorphism analysis: Genomic DNA was prepared from leukocytes. The resistin (*RETN)* -420 C/G (rs1862513) and the 3´ UTR C/T (rs10401670) polymorphisms were genotyped using custom allelic discrimination TaqMan® assays (C-

1394112-10 and C-1394125-10, respectively, Applied Biosystems) in a 7500 Fast Real-Time PCR System (Applied Biosystems).

Statistical analysis: Statistical analyses were performed using the SPSS software package, version 9.0 (SPSS, Inc. Chicago, IL). The results are expressed as mean (95% confidence interval). Allele frequencies were calculated by the gene counting method. A chi-square test was used to assess differences in genotype and allele frequencies. Differences in resistin levels between genotype were evaluated by one-factor ANOVA.

Results

The rs1862513 and rs10401670 polymorphisms were determined in 1269 and 1251 children, respectively. The genotype distributions for the two polymorphisms were similar to those previously reported for other Caucasian populations (Table 1). The prevalence for the less common allele G for the -420 C/G *RETN* polymorphism (rs1862513) was 32% and the frequency of allele T for the 3´UTR C/T *RETN* polymorphism (rs10401670) was 40%.

Resistin levels were measured in 342 prepubertal children and 690 12- to 16-year-old children. The SNP rs10401670 was associated with resistin levels in 6- to 8-year-old children, with carriers of the CT and TT genotypes showing resistin levels that were significantly higher than those of the CC carriers (Table 2). Although TT carriers seem to present higher resistin levels than CT or CC carriers among 12- to 16-year-old children, no significant differences were observed. The rs10401670 SNP was associated with BMI only in 12- to 16-year-old girls, with CT carriers showing significantly lower BMI and z-score BMI than CC carriers. No differences between the genotype distribution in normal-weight and overweight children were observed (data not shown).

When analyzing the relationship between the rs10401670 SNP and the lipid profile using ANOVA, we observed that 12- to 16-year-old male carriers of the T allele (CT and TT genotypes) had significantly higher total cholesterol and LDL-cholesterol levels than carriers of the CC genotypes (Table 3). In girls, the TT genotype was associated with significantly higher HDL-C levels.

No significant associations were found when analyzing the association of the rs1862513 with BMI, resistin levels, or lipid profile in boys or girls at any age.

Resistin levels were significantly correlated with Apo AI levels in 12- to 16-year-old normal-weight boys and girls.

Discussion

Despite the increasing amount of information regarding the association of resistin with metabolic alterations, the relationship between resistin polymorphisms and resistin levels, the anthropometric variables and obesity-related alteration remains unexplored in children. In our study analyzing two common *RETN* polymorphisms (rs1862513 and rs10401670) in healthy Spanish children, we have found a significant association between the rs10401670 SNP and resistin levels and lipid profile that happens to be different in girls and in boys and varies according to age.

The presence of the rs10401670 T allele is associated with significantly higher resistin levels in 6- to 8-year-old children, and even though the same tendency was observed in 12- to 16-year-old children, the differences did not reach statistical significance. To our knowledge, no studies have investigated resistin polymorphisms in children. Several studies in adult populations have described the association of SNPs in the resistin gene with variations in resistin concentration [18-22]. It also appears that the only study that has analyzed the effect of the rs10401670 polymorphism has found a strong association between the minor allele and higher resistin levels [23].

We have previously reported an association between resistin levels and body fat mass, but not BMI [32]. Studying the relationship of the SNPs rs1862513 and rs10401670 in the resistin gene with anthropometric variables, we did not observe any association of the rs1862513 with overweight or BMI, and only an inconsistent association of the rs10401670 SNP with BMI and z-score BMI in 12- to 16-year-old girls. Although no studies have analyzed the association of the rs10401670 SNP with anthropometric variables, contradictory results can also be found in previous studies of other polymorphisms in the resitin gene in adults [24-26, 28, 33, 34]. The study by Cieslak et al. [29], which examines the relationship of the several polymorphisms in the resistin gene with obesity in Polish children, failed to find

any significant association. Unfortunately, we do not have data on body fat composition from a large enough sample so as to analyze the association of these SNPs with body fat mass.

Resistin levels have been related with lipid profile in adults [4-11]. HDL-cholesterol levels have been reported to be significant predictors of resistin levels [35], with resistin being negatively correlated to HDL-C [6, 7, 10]. In our population we have found a negative correlation between resitin and ApoAI levels in normoweight 12- to 16-year-old children that was not observed in their overweight counterparts or in 6- to 8-year-old children. No association between HDL-C and resistin levels was found in the study by Rubin et al. [15] analyzing 10- to 14-year-old children, although the authors did not analyze normal-weight and overweight children separately. The study by Boyraz et al. [16] analyzing the relationship of resistin levels with metabolic syndrome components in obese children found a positive correlation between resistin and HDL-C levels, but the authors included a wide age-range in the population, studying children between 8 and 18 years. All these data suggest an age-dependent relationship between resistin and lipid profile.

An additional noteworthy finding in our study is the association between the rs10401670 and LDL-C levels in 12- to 16-year-old boys, and between the polymorphism and HDL-C levels in girls. As discussed previously, we have not found studies analyzing the effect of resistin polymorphisms in lipid profile in children. The studies in adults have reported inconsistent results [6, 17, 19, 22, 25, 27, 36, 37]. The study by Hivert et al. [23] analyzing the association of the rs10401670 SNP with diabetes-related traits in the Framingham Offspring Study did not include the relationship of the polymorphism with lipid variables in its analysis. It has been reported that resistin has a direct impact on human hepatic lipid and lipoprotein regulation, stimulating hepatic overproduction of atherogenic ApoB-containing lipoprotein particles by enhancing Apo B stability [38].

In conclusion, in our study in healthy children, we describe an age- and sex-specific association of the rs10401670 SNP with resistin concentrations and lipid levels, although the association of the polymorphism with anthropometric variables is weak. Our data suggest that this association may be influenced by the sex steroid levels associated with differences in age and gender.

Acknowledgements

The article is dedicated to the late Prof. Manuel de Oya as the warmest homage to his memory. Prof. de Oya designed the Four Province Study and the ideas reflected in our work can be traced back to his.

This work was supported by a grant from the *Fondo de Investigación Sanitaria* (FIS 11/00344) and Biobank grant FEDER RD09/0076/00101. The contract of C Garcés is co-financed by the *Fondo de Investigación Sanitaria.* Lorena Ortega is a fellow of the Conchita Rábago Foundation. We thank Oliver Shaw for his revision of our manuscript.

Table 1. SNPs 1862513 and rs10401670 genotype and allele frequencies.

rs1862513		rs10401670	
CC	46% (n=583)	CC	35.8% (n=447)
CG	44% (n=560)	CT	48.8% (n=610)
GG	10% (n=126)	TT	15.4% (n=194)
C	68%	C	60%
G	32%	T	40%

Table 2. Resistin levels (pg/ml) by SNP rs10401670 genotypes.

6- to 8-year-old boys			6- to 8-year-old girls		
CC (54)	*CT* (86)	*TT* (25)	*CC* (71)	*CT* (85)	*TT* (21)
2450	2688	2872	2346	2708	2884
(2217-2683)	(2511-2866)	(2471-3273)	(2154-2538)	(2508-2908)	(2506-3262)

12- to 16-year-old boys			12- to 16-year-old girls		
CC (111)	*CT* (167)	*TT* (58)	*CC* (129)	*CT* (173)	*TT* (52)
2311	2342	2404	2317	2384	2511
(2140-2481)	(2219-2465)	(2164-2644)	(2166-2469)	(2246-2522)	(2259-2763)

Table 3. Lipid profile by SNP rs 10401670 genotypes.

	12- to 16-year-old boys			12- to 16-year-old girls		
	CC (134)	*CT* (190)	*TT* (66)	*CC* (155)	*CT* (217)	*TT* (65)
CT (mg/dl)	**155.5**	**163.9**	**166.6**	168.4	172.8	168.9
	(150.9-160.1)	**(159.9-168.0)**	**(159.5-173.6)**	(164.0-172.8)	(168.8-176.8)	(161.8-175.9)
TG (mg/dl)	82.2	79.4	75.5	77.8	74.5	75.6
	(75.7-88.7)	(74.3-84.4)	(67.9-83.1)	(73.6-82.0)	(70.9-78.0)	(68.4-82.8)
LDL-C (mg/dl)	**90.4**	**97.6**	**99.8**	100.4	102.6	96.3
	(86.3-94.5)	**(94.0-101.3)**	**(94.3-105.4)**	(96.4-104.5)	(98.8-106.3)	(90.0-102.6)
ApoB (mg/dl)	67.8	69.9	70.2	71.9	70.6	69.0
	(65.2-70.4)	(67.5-72.3)	(65.9-74.5)	(69.4-74.5)	(68.5-72.8)	(65.2-72.8)
HDL-C (mg/dl)	48.6	50.4	51.6	**52.4**	**55.2**	**57.4**
	(46.2-51.0)	(48.5-52.4)	(47.4-55.9)	**(50.3-54.5)**	**(53.2-57.2)**	**(53.8-61.0)**
ApoAI (mg/dl)	140.8	146.6	143.2	144.9	149.2	149.4
	(136.5-145.2)	(142.8-150.5)	(136.7-149.7)	(141.4-148.5)	(145.7-152.6)	(142.8-156.1)

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