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Research

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Reward Deficiency Syndrome in Children: Obesity and Metabolic Disorders are Associated with the SNP TaqIA C32806T of the DRD2 Gene

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ABSTRACT

Background: Reward Deficiency Syndrome (RDS) is a hypo-dopaminergic state that predisposes to obsessive-compulsive behaviors. Obesity is part of RDS since the individual is predisposed to reward-driven eating behavior that leads to overeating. The allele A1 of the SNP C32806T in Dopamine D2 receptor gene (*DRD2*) is associated with reduction of *DRD2* levels and higher BMI in adults. *DRD2* are expressed in beta cells and modulate insulin secretion. The aim of this study is to investigate the relation between this SNP and obesity and metabolic alterations in children.

Methods: Fifty five obese children and 50 healthy controls were analyzed for *DRD2 Taq*1A polymorphism Genotyping was performed by polymerase chain reaction and restriction fragment length polymorphism. Glucose, insulin and lipid profile were measured. The Homeostatic model assessment (HOMA) was calculated.

Results: We found three genotypes: A1A1(12,4%), A1A2(33,3%) and A2A2(54,3%). The A1 allele was more present in: obese than in euthrophic (34,5%*23%), in children with altered HOMA ß (38,2% * 24,6%), children with altered Total Cholesterol (35,2%*19,5%) and lower levels of triglycerides. Children were divided in 4 subgroups in accordance to the function of pancreatic beta cells and BMI-Z; subgroups with normal secreting pancreatic beta cell demonstrated significant difference for allelic and genotypic distribution, with lower presence of A1A1 and A1A2 genotypes and higher presence of A2 allele.

Conclusions: Besides confirming the association with childhood obesity, our results show for the first time that: A1 allele is associated with TC≥170 mg/dl, lower TG levels and HOMA ß ≥175. A2 allele is associated with normal HOMA ß, being a protective factor for pancreatic secretion. The recognition of predisposed individuals through determinations of risks polymorphisms can lead to new paths for treatment and prevention of metabolic abnormalities.

KEYWORDS: Childhood obesity; DRD2 gene; HOMA; Dopamine; Genetic polymorphism.

ABBREVIATIONS: RDS: Reward Deficiency Syndrome; DRD2: Dopamine D2 receptor gene; WHO: World Health Organization; PET: Positron Emission Tomography; HOMA: Homeosta-

sis model assessment; RFLP-PCR: Restriction fragment length polymorphism system polymerase chain reaction; OR: Odds Ratio; ANOVA: Analysis of variance; DM2: Type 2 Diabetes Mellitus; ADHD: Attention Deficit/Hyperactivity Disorder.

INTRODUCTION

Exogenous obesity is a complex disease of multifactorial etiology in which pleiotropic genetic syndromes and monogenic diseases account for only 1% of cases. It is recognized by World Health Organization (WHO) as one of the ten most important health problems in many societies.[1] The prevalence of obesity has grown worldwide, being endemic in several developed and transition countries, and it is an important cause of morbidity and mortality in the developing world.[2] A report published in January 2014 conducted by the Overseas Development Institute in Britain shows a general picture of the evolution of the obesity in the world in the last 30 years. Adult overweight is observed in 70% of North American's, and in 63% of Latin American's, a significant increase compared to 30% observed in the 80's.[3]

About 60% of obese children, aged 5-10 years have at least one risk factor for cardiovascular disease (hypertension, dyslipidemia, hyperinsulinemia, impaired glucose metabolism, thrombotic risk factors) and 20% of these children have two or more of these factors.[4] In addition to metabolic complications, the obese children and adolescents also have higher incidence of asthma, sleep apnea, polycystic ovary syndrome, and psychosocial complications.[5]

Studies show that heredity is an important factor for childhood-onset obesity.[6,7] The largest study concerning the genetics of childhood obesity, where 5530 cases and 8318 controls were evaluated, showed a strong genetic influence in the development of childhood obesity.[8]

The dopamine type-2 receptor *(DRD2)* gene contains 66.097 pb, it is located on chromosome 11 (q22-q23) and encodes the D2 subtype of the dopamine receptor, a transmembrane protein that couple to G-protein and inhibits adenylyl cyclase activity. This gene was included in HOGM (Human Obesity Gene Map) supported by 5 studies of candidate genes, but none of these studies had included children's evaluation.[9]

The *DRD2* gene is highly polymorphic, and therefore, there are several SNPs described for it. The C32806T SNP, a C-T substitution located in a noncoding region of the *DRD2* locus, affects the availability of the D2 receptor. The A1 allele (T) has been associated with reduced glucose metabolic rate in dopaminergic human brain regions.[10] Variations on dopaminergic receptors and in dopamine release are involved with overeating and obesity. When exposed to high-fat diet, mice with decreased levels of *DRD2* gain more weight than mice with normal levels.[11] Studies have suggested that obese may have a decreased availability of dopamine by a striatal D2 dopaminergic receptors down-regulation mechanism. Drugs that block these receptors increase appetite and drugs that increase the central dopamine concentration have anorectic effects.[12]

Several studies in adults suggest that increases of body mass are associated with *DRD2* A1 allele,[13-15] and mutations in this gene have also been associated with schizophrenia and alcoholism.[14-16] Positron Emission Tomography (PET) studies showed that A1 allele is associated with lower *DRD2* density,[17] and reduction of glucose metabolism in dopaminergic human brain regions.[18]

The reward deficiency syndrome (RDS) is a hypo-dopaminergic state that predisposes to obsessive-compulsive and impulsive behaviors.[19,20] Obesity is part of RDS since the individual is predisposed to reward-driven eating behavior that leads to overeating as a way to compensate the defect in dopamine levels. All components of RDS, including obesity, were related to low dopaminergic function associated with the presence of the *DRD2* A1 allele.[13,19,20] Thus, the aim of this study was to investigate the association between *DRD2* gene *Taq*1A polymorphism and obesity, dyslipidemia and insulin resistance in children.

MATERIAL AND METHODS

Subjects

This study was conducted with 105 children and adolescents aged between 5 and 16 years (55 obese and 50 normal-weight controls) that were evaluated by a pediatric endocrinologist at Children's Hospital Goiânia. Exclusion criteria were: overweight, malnutrition, severe chronic diseases, presence of genetic syndromes, use of medications that can alter weight (glucocorticoids, growth hormone, insulin, Gonadotropin-releasing hormone-GnRH analogues, etc.).

Parents or guardians provided informed consent prior to participation in the study. Consent was approved by the Ethics Committee on Human Research at the Pontifical Catholic University of Goiás, under the protocol number 16303313.4.0000.0037. Parents or guardians answered a questionnaire focusing on lifestyle and habits of the child and family, the presence of obesity related diseases (diabetes mellitus, hypertension, dyslipidemia, myocardial infarction, and stroke), informed the height and maximum weight of each parents, excluding maternal pregnancy period, for calculation of maximum Body Mass Index (BMI) of parents.

The diagnosis of nutritional status was based on the BMI according to WHO.[21] Anthropometric data (weight and height) were calculated based on the WHO Anthro Plus software (http://www.who.int/childgrowth/software/en/.48). Itran the BMI calculation in absolute number and in Z score according to age and sex of each child. We also evaluated metabolic measurements including lipid profile, fasting glucose and insulin. The homeostasis model assessment (HOMA) for insulin resistance

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(HOMA-IR) and for beta cell function (HOMA ß) were calculated using traditional formulas HOMA-IR=(fasting insulin [µU/ml] * fasting glucose [mmol/l]/22.5) and HOMA ß=(20*fasting insulin [µU/ml] * fasting glucose [mmol/l] – 3.5), the reference values are ≤3.4 and 167 to 175, respectively.[22]

Reference values for fasten glucose (mg/dl) do not differ between adults and children, being normal from 60 to 100, pre-diabetic between 101 and 125 and diabetic ≥126. Fasten insulinemia (UI/ml) is considered normal from 2,5 to 25 in adults and until 15 in children.[23] Lipidogram was evaluated according to the I Brazilian Guidelines for Atherosclerosis Prevention in children and adolescents,[24] as follows in Chart 1:

Lipid levels	Desirable (mg/dl)	Frontier (mg/dl)	Elevated (mg/dl)
Total Cholesterol (CT)	<150	150-169	≥170
Low-density lipoprotein(LDL-C)	<100	100-129	≥130
High-densitylipoprotein(HDL-C)	≥45		
Triglycerides (TG)	<100	100-129	≥130

Chart 1: Referencesvalues for lipidlevels for chidrenfrom 2-19 year according to the I Brazilian Guidelines for Atherosclerosis Prevention in children and adolescents.

Genotyping

Genotyping was performed to identify the polymorphism *Taq*1A (C32806T or rs1800497) of *DRD2*. Biological samples were obtained by collecting 5 ml of peripheral blood in EDTA. Genomic DNA was isolated from whole blood using Illustra blood genomic Prep Mini Kit (GE Healthcare, United Kingdom) according to manufacturer's instructions. DNA quantification was carried out using a NanoVue™ Plus spectrophotometer (GE Healthcare, United Kingdom) and stored in -20 °C until further processing.

To evaluate the *DRD2* polymorphism we performed Restriction fragment length polymorphism system polymerase chain reaction (RFLP-PCR) according to Jönsson, et al.[17] Amplification reactions were prepared to a final volume of 50 µL of solution containg approximately 100 ng of DNA. The reactions was prepared with 2 mM of $MgCl_2$, 50 mM of KCl, 15 mM of Tris-HCl (pH 8.4), 10 pmol of primers, 0.2 mM of each dNTPs, and 1 U of *Taq* DNA polimerase (Promega Coorporation, EUA). The thermocycling protocol was: initial denaturation of 95 °C for 3 min followed by 30 cycles of denaturation at 95 °C for 30 seconds, annealing at 58 °C for 30 seconds and extension at 72 °C for 60 seconds, and the extension at 72 °C for 5 min. The sequence of the primers used in the RFLP-PCR reaction were MP3 primer (5'- ACCCTTCCTGAGTGTCATCA-3') and MP4 primer (5'- ACGGCTGGCCAAGTTGTCTA-3') producing an amplicom of 310 pb.

The restriction enzyme was carried out using a final volume of 25 µL, containing 8 µL of the PCR product, 2,5 µL of buffer, and 1 unit of the enzyme *Taq*IA. The solution remained for 1 hour at 65 °C in a water bath. The restriction resulted in two fragments, one of 130 bp and another of 180 bp.

The amplicons and the restriction fragments were visualized by 1,5% agarose gel electrophoresis, submitted to an electric field of 10 V/cm, for one and a half hour. The PCR products were stained with ethidium bromide 0.5 mg/mL and documented using the Video Documentation System (VDS®, Amersham Bioscience, EUA), connected to a microcomputer with a capture system and analysis of 21 images (Imagemaster®, Amersham Bioscience, EUA).

Individuals with genotype A1/A1 (TT) do not have the site for the restriction enzyme *Taq*IA, therefore producing only undigested fragments of 310 pb. Individuals with genotypes A2/A2 (CC) after the action of restriction enzyme *Taq*IA produce two fragments, 180 pb and 130 pb. Finally, individuals with heterozygous genotype A1/A2 (CT) show three fragments, 310 pb, 180 pb and 130 pb.

Statistical Analysis

Metabolic measurements, HOMA IR, HOMA ß, parents BMI, and child Z-BMI were correlated with polymorphism using SPSS17 *Statistics* software. Data was analyzed with the X2 test, Odds Ratio (OR) and Analysis of variance (ANOVA). All tests were considered statistically significant at p≤0.05 with 95% of confidence interval.

RESULTS

Anthropometric and clinical analysis revealed 55(52,4%) obese and 50(47,6%) eutrophic children. In the first group, 28(50,9%) were female and 27(49,1%) were male, and in the second group 27(54%) were female and 23(46%) were male. Both groups were similar to age and gender. There were significant differences (p≤0.05) between the two groups for weight, height, BMI-Z, parents BMI, insulin, HOMA-IR, HOMA- ß, and HDL-C (Table 1).

Genotypic distribution of *DRD2 Taq*1A (C32806T or rs1800497) was: 12,4% A1A1, 33,3% A1A2, and 54,3% A2A2. This distribution showed higher proportion to A1A1 and A1A2 in the obese group, and A2A2 in the eutrophic group, but this was not significantly different. Allelic distribution was significantly different (p=0.05) and there was a 1.3 RR for obesity in those who carried the A1 allele (Table 2).

Children were evaluated in groups according to the presence of allele A1, the A1A1+A1A2 group being the "risk genotype" and the A2A2 group providing the "no risk genotype". This analysis showed statistical significance for weight, BMI-Z, mother BMI and TG (Table 3). The evaluation of genotypic distribution showed significant differences for BMI-Z, father BMI and TG (Table 4).

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	Obese		Eutrophic		
Characteristics	**Median**	**±SD**	**Median**	**±SD**	***p***
Age (Years)	9,6	1,8	10,2	2,3	0,194
Weight (kg)	56,6	15,6	30,2	7,6	<0,0001*
Hight (cm)	142,5	10,2	135,5	12,9	0,013*
Z BMI	3,19	0,9	- 0,6	0,6	<0,0001*
Mother BMI (kg/m^2)	29,2	5,3	23,46	2,6	<0,0001*
Father BMI (kg/m2)	31,88	4,6	26,18	2,9	<0,0001*
Fasten Glucose (mg/dl)	84,82	5,2	86,02	5,7	0,362
FastenInsulin (Ul/ml)	12,44	5,8	6,37	2,4	<0,0001*
Homa IR	2,64	1,3	1,36	0,5	<0,0001*
Homa ß	212,23	96,5	107,34	43,7	<0,0001*
TC (mg/dl)	167,05	24,3	165,7	19,7	0,809
HDL-C (mg/dl)	40,44	5,7	48,98	8,4	<0,0001*
LDL-C (mg/dl)	109,62	20,9	101,2	18,4	0,101
TG (mg/dl)	86,73	31,0	80,06	29,4	0,388
Total	55(100)		50(100)		

BMI: Body Mass Index; Z BMI: Z score for BMI; HOMA: Homeostasis model assessment; IR: Insulin Resistance; ß: beta cell function; TC: Total Cholesterol; HDL-C: High-density lipoprotein; LDL-C: Low-density lipoprotein; TG: Triglycerides; SD: Standard Deviation.

Table 1: Anthropometric and clinical characteristics of obese and eutrophic groups.

AllelicDistribution	**ObeseGroup*n* (%)**	**EutrophicGroup*n* (%)**	**RR**	***p***
A1	38(34,5)	23(23)	1,2892	(0,05)*
A2	72(65,5)	77(77)		
Total	110(100)	100(100)		

RR: Relative Risk.

Table 2: Allelic distribution of obese and eutrophic groups.

	RiskGenotype A1A1 + A1A2		**Non-risk Genotype A2A2**		***p***
Variables	**Median**	**±SD**	**Median**	**±SD**	
Years (months)	119,6	29,85	118,3	31,24	0,823
Weight (Kg)	48,86	21,66	40,01	19,64	0,03*
Hight (cm)	141,5	12,05	137,3	16,08	0,135
Z BMI	1,97	4,18	0,88	2,06	0,009*
Mother BMI (kg/m^2)	27,87	7,7	25,32	4,18	0,05*
Father BMI (kg/m^2)	29,91	7,33	28,57	4,64	0,273
Fasten Glucose (mg/dl)	85,29	6,78	85,47	6,69	0,891
FastenInsulin (Ul/ml)	10,39	6,88	8,85	7,39	0,274
Homa IR	2,19	1,48	1,89	1,68	0,33
Homa ß	182,19	121,49	145,51	103,96	0,098
TC (mg/dl)	161,1	26,2	170,88	29,8	0,08
HDL-C (mg/dl)	73,62	29,12	91,91	44,71	0,013*
LDL-C (mg/dl)	44	7,98	44,93	11,13	0,63
TG (mg/dl)	102,79	23,65	107,98	28,25	0,315
Total	48(100)		57(100)		

BMI: Body Mass Index; Z BMI: Z score for BMI; HOMA: Homeostasis model assessment; IR: Insulin Resistance; ß: beta cell function; TC: Total Cholesterol; HDL-C: High-density lipoprotein; LDL-C: Low-density lipoprotein; TG: Triglycerides; SD: Standard Deviation.

Table 3: Anthropometric and clinical characteristics in the observed risk genotype group (A1A1+A1A2) and non-risk genotype group (A2A2).

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	Genotype				
	A1A1	A1A2	A2A2		
Variables	MedianMedianMedian			F	*p*
Z BMI	1,948	1,977	0,88	3,470	0,035*
Mother BMI (kg/m²)	27,66	27,95	25,32	2,178	0,119
Father BMI (kg/m²)	33,29	28,65	28,58	3,385	0,038*
Glucose (mg/dl)	84,92	85,43	85,47	0,036	0,965
Insulin (UI/ml)	11,2	10,08	8,85	0,715	0,491
HOMA IR	2,346	2,14	1,89	0,553	0,577
HOMA ß	201,96	174,85	145,51	1,659	0,195
TC (mg/dl)	153	164,11	170,88	2,308	0,105
HDL-C (mg/dl)	40,07	45,46	44,93	1,568	0,213
LDL-C (mg/dl)	97,15	104,89	107,98	0,919	0,402
TG (mg/dl)	82,84	70,2	91,91	3,471	0,035*
	N (%)				
TOTAL	13(100)	35(100)	57(100)		

BMI: Body Mass Index; Z BMI: Z score for BMI; HOMA: Homeostasis model assessment; IR: Insulin Resistance; ß: beta cell function; TC: Total Cholesterol; HDL-C: High-density lipoprotein; LDL-C: Low-density lipoprotein; TG: Triglycerides; SD: Standard Deviation.

Table 4: Anthropometric and clinical characteristics observed in the genotype groups.

Allelic distribution was compared with groups classified according to the references values for normality of TC (<170 mg/dl-normal range or ≥170 mg/dl-elevated level)[22] and HOMA ß (<175-normal or ≥175-altered).[24] We observed significant difference for allelic distribution in children with TC<170 mg/dl or ≥170 mg/dl and in children with HOMA ß<175 or ≥175. The RR for A1 allele was 1.5 for HOMA ß≥175 (Tables 5 and 6). Associations between the A1 allele and TC ≥170 mg/dl and HOMA ß≥175 have not been described in the literature until now.

Allele	TC < 170(mg/dl) *n*(%)	TC ≥ 170 (mg/dl) *n*(%)	*p*
A1	16(19,5)	45(35,2)	0,0249*
A2	66(80,5)	83(64,8)	
Total	82(100)	128(100)	

Table 5: Allelic Distribution in the patients with TC<170 and TC≥170.

Allele	HOMA ß < 175 *n*(%)	HOMA ß ≥ 175 *n* (%)	RR	*p*
A1	35(24,6)	26(38,2)	1,5121	0,0367*
A2	107(75,4)	42(61,8)		
Total	142(100)	68(100)		

RR: Relative Risk.

Table 6: Allelic Distribution in the patients with HOMA ß< 175 and HOMA ß≥175.

Children were divided in 4 subgroups in accordance to the function of pancreatic beta cells and BMI-Z: obese with normal HOMA ß (OßN), obese with altered HOMA ß (Oß↑), eutrophic with normal HOMA ß (EßN) and eutrophic with altered HOMA ß (Eß↑). Twenty six (47,3%) and 29(52,7%) obese children presented normal HOMA ß and altered HOMA ß, respectively. In the eutrophic group 45(90%) presented normal index and 5(10%) altered index. Allelic and genotypic distribution is shown in Tables 7, 8, 9 and 10. Subgroups with normal secreting pancreatic beta cell (OßN e EßN) demonstrated significant difference for allelic and genotypic distribution, with lower presence of A1A1 and A1A2 genotypes and higher presence of A2 allele.

	Obese					
	HOMA ß<175 (OßN)			HOMA ß≥175 (Oß↑)		
Genotype	*n*(%)	X²	*p*	*n* (%)	X²	*p*
A1 A1	2(7,7)	7,923	0,019*	6(20,7)	2,138	0,343
A1 A2	11(42,3)			11(37,9)		
A2 A2	13(50)			12(41,4)		
Total	26(100)			29(100)		
TOTAL	55(100)					

Table 7: Genotypic distribution in the obese subgroups with normal secreting pancreatic beta cells (OßN) and altered secreting pancreatic beta cells (Oß↑).

	Eutrophic					
	HOMA ß<175(EßN)			HOMA ß≥175(Eß↑)		
Genotype	*n*(%)	X²	*p*	*n*(%)	X²	*p*
A1 A1	4(8,9)	21,733	<0,0001*	1(20)	1,6	0,449
A1 A2	12(26,7)			1(20)		
A2 A2	29(64,4)			3(60)		
Total	45(100)			5(100)		
TOTAL	50(100)					

Table 8: Genotypic distribution in the eutrophic subgroups with normal secreting pancreatic beta cells (EßN) and altered secreting pancreatic beta cells (Eß↑).

	Obese					
	HOMA ß<175(OßN)			HOMA ß≥175(Oß↑)		
Allele	*n*(%)	X²	*p*	*n* (%)	X²	*p*
A1	15(28,8)	8,481	0,0036*	23(39,7)	2,09	0,1486
A2	37(71,2)			35(60,3)		
TOTAL	52(100)			58(100)		

Table 9: Allelic distribution in the obese subgroups with normal secreting pancreatic beta cells (OßN) and altered secreting pancreatic beta cells (Oß↑).

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	Oß↑	EßN		
Allele	*n*(%)	*n*(%)	X²	*p*
A1	23(39,7)	20(22,2)		
A2	35(60,3)	70(77,8)	4,389	0,0362*
Total	58(100)	90(100)		

Table 10: Allelic distribution in the eutrophic subgroups with normal secreting pancreatic beta cells (EßN) and altered secreting pancreatic beta cells (Eß↑).

We compared allelic distribution between these 4 subgroups, and the groups Oß↑ and EßN were statistically different. Obese children with altered HOMA ß have a major presence of A1 allele (Table 11).

	Oß↑	EßN		
Allele	*n*(%)	*n*(%)	X²	*p*
A1	23(39,7)	20(22,2)		
A2	35(60,3)	70(77,8)	4,389	0,0362*
Total	58(100)	90(100)		

Table 11: Comparison of allelic distribution in the Oß↑ e EßN subgroups.

DISCUSSION

Anthropometric and clinical analysis revealed some differences between the obese and eutrophic groups. The significant difference in weight and BMI-Z is expected and is itself the definition of each group. The greater height observed in the obese group is in accordance with usual clinical findings in this group of children, and is confirmed by clinical studies showing that obese children are taller than their peers of same age. However, since they have accelerated bone maturation, their growth occurs for a shorter period of time, not leading to changes in final adult height.[25]

The presence of higher levels of parents BMI of obese children corroborates studies that indicate a strong genetic component in human obesity.[26-28] The cchildren in the present study coexist in the same environment as their biological family (except for two), and therefore are exposed to the same lifestyle. It's known that a child who has both obese parents has 80% chance of being obese, 50% if one parentis obese, and 9% when both parents are not obese.[29] In our study, the mean BMI of parents is above normal in both groups. The eutrophic children group had overweight parents, consistent with the increase of overweight and obesity in the adult Brazilian population, reaching 48% of women and 50% of men.[30]

In this study, obese and eutrophic groups presented the same level of blood glucose, however, obese group showed higher levels of insulin, HOMA IR and HOMA ß. These changes in insulin sensitivity and pancreatic beta cell secretion are related to obesity, which is an important risk factor for the development of Type 2 Diabetes Mellitus (DM2).[4] The finding of lower level of HDL-C in the obese group is expected, since the presence of risk factors for cardiovascular disease in obese children is common.[4,5]

The *Taq*IA polymorphism (C32806T) of *DRD2* gene is associated with decreased brain dopaminergic activity,[31] and the A1 allele was related with increased BMI in adults.[20,32] Few studies have been conducted to verify the association of the *DRD2* polymorphism *Taq*IA in children and adolescents.[33]

In this study, we identified 34,5% and 23% for A1 allele in obese and eutrophic groups respectively, and a statistically significant association between the presence of A1 allele with childhood obesity conferringa relative risk of 1,3. Studies noted a wide variation in allele frequencies, even within the same country populations. In two studies conducted with Turkish children, one of them showed 51% of the A1 allele in obese,[34] while another reported only 20%.[23] A Dutch study showed the frequency of 18.3% of A1 allele[35] and in North American studies the frequency of the A1 allele in obese children ranged from 17%[36] to 38.5%.[37]

The *DRD2 Taq*IA polymorphism was previously evaluated in children and/or adolescents among few other groups. The A1 allele was associated with obesity in one study,[37] other 2 studies did not find this association;[23,34] it was also described compulsive feeding behavior[35] and difficulties in acceptance for healthier life style modification.[36]

Although TG levels were lower in the A1 allele group, this difference was not clinically relevant, since all groups showed TG in the normal range. TG levels were 20% lower in the "risk genotype" group as compared with "no risk genotype" group and was 10% lower in subjects with A1A1 genotype compared with A2A2 genotype group. Miyashita and colleagues performed a meta-analysis evaluating the effect of physical exercise on TG levels and found significant decreases in TG after short time and intermittent physical exercise. The decrease in TG levels ranged from 10% to five minutes practice of exercise six times per day, up to 27% for practice of 10 minutes 3 times per day.[38] It was hypothesized that children with the presence of the A1 allele have the lowest TG, due to higher levels of physical activity. The presence of A1 allele contributes to the Reward Deficiency Syndrome (RDS), in which Attention Deficit/Hyperactivity Disorder (ADHD) is part,[20] moreover studies assessing the *Taq* IA polymorphism of *DRD2* gene and personality traits using the Tridimensional Personality Questionnaire demonstrate that A1 allele is associated with certain behavioral traits, with higher levels of impulsivity, extravagance, disorganization,[39-42] persistence[39-41] and gregarious attitude.[40-42] These behavioral characteristics may lead to greater practice of physical exercise by the children with the A1 allele, and thus may explain lower TG levels.

The association of A1 allele with higher frequency of CT≥170 mg/dl is explained by the feeding behavior assigned to A1 allele carriers as a way to compensate for the RDS.

Our study demonstrated the association of impaired

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function of pancreatic beta cells with A1 allele conferring a relative risk of 1.5 for HOMA ß≥175. Clinical studies in diabetic patients[43] and in animal studies[44] show improvement of glucose control with the use of bromocriptine, a dopamine agonist which acts throw *DRD2* receptors. In 2005, Ruby and colleagues first demonstrated that *DRD2* receptors are expressed in pancreatic beta cells and modulate the secretion of insulin.[45] Study conducted in rats "knockout" for the *DRD2* gene revealed that *DRD2* receptors play a crucial role in insulin secretion and glucose homeostasis; mice with absence of the *DRD2* receptor had a flat insulin response to glucose load, higher fasting glucose, impaired glucose tolerance and decreased beta cell mass.[45] These results indicate that *DRD2* is important for beta cell proliferation and insulin secretion, and may be considered as a growth factor essential for the control of glucose homeostasis.[46]

For the first time, the A1 allele of the *DRD2* gene is associated with alteration of glucose homeostasis in humans. The presence of this allele reduces the number of brain receptors; it is assumed that must also reduce the number of receptors on beta cells, explaining our clinical findings. The presence of the A2 allele was associated with normal HOMA ß in eutrophic and obese patients, demonstrating a protective effect of this allele in the pancreatic secretion.

CONCLUSIONS

Regarding the *Taq*IA (C32806T) polymorphism of *DRD2* gene we observed several statistically significant results. A1 allele (T) is associated with: higher weight and children Z-BMI, conferring a relative risk of 1.3 for the presence of childhood obesity. This allele is also associated with higher BMI of the mother and father. Regarding the lipid profile, the A1allel-eis associated with lower levels of TG and higher frequency of CT≥170 mg/dl.

With respect to carbohydrate metabolism, our study found an unprecedented result in the literature: the A1 allele was associated with HOMA ß≥175, giving a relative risk of 1.5. The A2 allele was associated with normal HOMA ß in obese and eutrophic individuals, demonstrating the involvement of this allele as a protective factor for pancreatic secretion.

The major limitation of this study is the relatively small population. Even though, our research opens a new line of investigation: the relationship between early-onset obesity, the *Taq*IA polymorphism of the DRD2 gene and abnormality of secretion of pancreatic beta cells

Despite widely recognized that genetic factors are important for weight gain, the actual quantitative contribution of genetics in related phenotypes is still a complex issue that needs to be clarified. The knowledge of the genetic architecture of obesity will increase our understanding of the regulation of energy balance in humans, and therefore, provide new paths for the treatment and prevention of this serious health problem. The recognition of predisposed individuals by determining risk polymorphisms can establish new pathways for the treatment and prevention of childhood obesity. We believe that in the future children will be treated based on their genomes.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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REFERENCES

1. World Health Organization. Obesity preventing and managing the global epidemic; Report of a WHO Consultation (WHO Technical report series 894). Geneve, WHO. 2000; 252.

2. Bell CG, Walley AW, Frogue lP. The genetics of human obesity. *Nature Reviews Genetics.* 2005; 6: 221-234. doi: 10.1038/nrg1556

3. Keats S, Wiggins S. Future diets: Obesity is on the rise globally. Website: http://www.odi.org.uk/future-diets, 2014.

4. Amemiya S, Dobashi K, Urakami T, Sugihara S, Ohzeki T, Tajima N. Metabolic syndrome in youths. *Pediatric Diabetes*. 2007; 8: 48-54. doi: 10.1111/j.1399-5448.2007.00226.x

5. Radominski RB. Epidemiological aspects of childhood obesity. *Revista ABESO*. 2011; 49: 10-13.

6. Demerath EW, Choh AC, Czerwinski SA, et al. Genetics and environmental influences on infant weight and weight change: the FELS Longitudinal Study. *Am J Hum Biol.* 2007; 19: 692-702.

7. Griffiths LJ, Dezateux C, Cole TJ. Differential parental weight and height contributions to offspring birthweight and weight gain in infancy. *Int J Epidemiol.* 2007; 36: 104-107. doi: 10.1093/ije/dyl210

8. Bradfield JP, Taal HR, Timpson NJ, et al. A genome-wide association meta-analysis identifies new childhood obesity loci. *Nature Genetics.* 2012; 44: 526-531. doi: 10.1038/ng.2247

9. Rankinen T, Zuberi A, Chagnon YC, Weisnagel SJ, Argyropoulos G, Walts B. The human obesity gene map: the 2005 update. *Obes Res.* 2006; 14: 529–644. doi: 10.1038/oby.2006.71

10. Neville MJ, Johnstone EC, Walton RT. Identification and characterization of ANKK1: a novel kinase gene closely linked to *DRD2* on chromosome band 11q23.1. *Hum Mutat.* 2004; 23: 540-545. doi: 10.1002/humu.20039

11. Huang XF, Zavitsanou K, Huang X, et al. Dopamine transporter and D2 receptor binding densities in mice prone or resistant to chronic high fat diet-incuded obesity. *Behav Brain Res.* 2006; 175: 415-419. doi: 10.1016/j.bbr.2006.08.034

12. Wang GJ, Volkow ND, Logan J, et al. Brain dopamine and obesity. The lancet. 2001; 3: 354-357. doi: 10.1016/S0140-6736(00)03643-6

13. Blum K, Braverman ER, Wood RC, et al. Increased prevalence of the *Taq* I A1 allele of the dopamine receptor gene (*DRD2*) in obesity with comorbid substance use disorder: a preliminary report. *Pharmacogenetics.* 1996; 6: 297-305.

14. Nisoli E, Brunani A, Borgomainerio E, et al. D2 dopamine receptor (*DRD2*) gene *Taq*1A polymorphism and the eating-related psychological traits in eating disorders (anorexia nervosa and bulimia) and obesity. *Eat Weight Disord.* 2007; 12: 91-95. 10.1007/BF03327583

15. Chen AL, Blum K, Chen TJ, et al. Correlation of the *Taq*1 dopamine D2 receptor gene and percent body fat in obese and screened control subjects: a preliminary report. *Food Funct.* 2012; 3: 40-48. doi: 10.1039/c1fo10089k

16. Kukreti R, Tripathi S, Bhatnagar P, et al. Association of *DRD2* gene variant with schizophrenia. *Neurosci Lett.* 2005; 392: 68-71. doi: 10.1016/j.neulet.2005.08.059

17. Jönsson EG, Nöthen MM, Gründhage F, et al. Polymorphisms in the dopamine D2 receptor gene and their relationships to striatal dopamine receptor density of healthy volunteers. *Mol Psychiatry.* 1999; 4: 290-296.

18. Noble EP, Gottschalk LA, Fallon JH, Ritchie TL, Wu JC. D2 dopamine receptor polymorphism and brains regional glucose metabolism. *Am J Med Genet B Neuropsychiatr Genet.* 1997; 7: 162-166. doi: 10.1002/(SICI)1096-8628(19970418)74:2<162::AID-AJMG9>3.0.CO;2-W

19. Blum K, Chen ALC, Giordano J, et al. The addictive brain: all roads lead to dopamine. *Journal of Psychoactive Drugs.* 2012; 44: 134-143. doi: 10.1080/02791072.2012.685407

20. Blum K, Cull J, Braverman ER, Comings D. Reward deficiency syndrome. *American Scientist.* 1996; 84: 132-145.

21. World Health Organization. WHO Expert Comitee on Physical Status: the use and interpretation of antropometry and physical status (WHO Technical Report Series, 854). Geneve, WHO. 1995; 452.

22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 1985; 28: 412-419.

23. Araz NC, Nacak M, Balci SO, et al. Childhood obesity and the role of dopamine D2 receptor and cannabinoid receptor-1 gene polymorphisms. *Genetic Testing and Molecular Biomarkers*. 2012; 16: 1408-1412. doi: 10.1089/gtmb.2012.0244

24. Brazilian Society of Cardiology. Atherosclerosis Prevention Guidelines in Childhood and Adolescence. *Brazilian Archives of Cardiology.* 2005; 85: 1-36.

25. Metcalf NS, Hosking J, Fremeaux AE, Jeffery AN, Voss LD, Wilkin TJ. BMI was right all along-taller childen really are fatter 3 implications of making childhood BMI independent of height. Int J Obes. 2011; 35: 541-547. doi: 10.1038/ijo.2010.258

26. Bouchard C, Perusse L, Rice T, Rao DC. The genetics of human obesity; In: Dekker M, ed. Handbook of Obesity, New York, Bray G, 1998; 157-190.

27. Rankinen T, Perusse L, Weisnagel SJ, Snyder EE, Chagnon YC, Bouchard C. The human obesity gene map: the 2001 update. *Obes Res.* 2002; 10: 196-243.

28. Hainer V, ZamrazilováH, Spálová J, et al. Role of Hereditary Factors in Weight Loss and Its Maintenance. *Physiol. Res.* 2008; 57: S1-S15.

29. Fisberg M, Cintra IP, Costa RF. Obese children and adolescents: epidemiology, diagnosis, body composition and treatment. In: Setian N, ed. Obesity in children and adolescents. São Paulo, Roca. 2007; 7-28.

30. Brazilian Institute of Geography and Statistics. Family Budget Survey (POF) 2008-2009- anthropometry and nutritional status of children, adolescents and adults in Brazil. In: de Janeiro R, ed. IBGE. 2010; 1-130.

31. Noble EP, Blum K, Ritchie T, Montgomery A, Sheridan PJ. Allelic association of the D2 dopamine receptor gene with receptor-binding characteristics in alcoholism. *Archives of general Psychiatry.* 1991; 48: 648-654. doi: 10.1001/archpsyc.1991.01810310066012

32. Stice E, Yokum S, Bohon C, Marti N, Smolen A. Reward circuitry responsivity to food predicts future increases in body mass: moderating effects of *DRD2* and DRD4. *Neuroimage.* 2010; 50: 1618-1625. doi: 10.1016/j.neuroimage.2010.01.081

33. Böettcher Y, Korner A, Kovacs P, Kiess P. Obesity genes:

ISSN 2377-8385 Open Journal http://dx.doi.org/10.17140/OROJ-2-111

implication in childhood obesity. *Pediatrics and child health.* 2011; 22: 31-36. doi: 10.1016/j.paed.2011.08.009

34. Ergun MA, Karaoguz MY, Koc A, et al. The apolipoprotein E gene and *Taq*1A polymorphisms in childhood obesity. *Genetic Testing and Molecular Biomarkers.* 2010; 14: 343-345. doi: 10.1089/gtmb.2010.0002

35. Strien T, Snoek HM, van der Zwaluw C, Engels RC. Parental control and the dopamine D2 receptor gene (*DRD2*) interaction on emotional eating in adolescence. *Appetite.* 2010; 54: 255-261. doi: 10.1016/j.appet.2009.11.006

36. Roth CL, Hinne A, Schur EA, Elfers CT, Reinehr T. Association analyses for dopamine receptor gene polymorphisms and weight status in a longitudinal analysis in obese children before and after lifestyle intervention. *BMC Pediatrics*. 2013; 13: 197-205. doi: 10.1186/1471-2431-13-197

37. Duran-Gonzalez J, Ortiz I, Gonzales E, et al. Association study of candidate gene polymorphisms and obesity in a young mexican-american population from South Texas. *Arch Med Research*. 2011; 42: 523-531. doi: 10.1016/j.arcmed.2011.10.010

38. Miyashita M, Burns SF, Stensel DJ. An update on accumulating exercise and postprandial lipaemia: translating theory into practice. *J Prev med Public Health.* 2013; 46: S3-S11. doi: 10.3961/jpmph.2013.46.S.S3

39. Lee HJ, Lee HS, Kim YK, et al. D2 and D4 dopamine receptor gene polymorphisms and personality traits in a young Korean population. *Am J Med Genet B Neuropsychiatr Genet.* 2003; 121B: 44-49. doi: 10.1002/ajmg.b.20054

40. Noble EP, Ozkaragoz TZ, Ritchie TL, Zhang X, Belin TR, Sparkes RS. D2 and D4 dopamine receptor polymorphisms and personality. *Am J Med Genetics.* 1998; 81: 257-267. doi: 10.1002/(SICI)1096-8628(19980508)81:3<257::AID-AJMG10>3.0.CO;2-E

41. Noble EP. D2 dopamine receptor gene in psychiatric and neurologic disorders and its phenotypes. *Am J Med Gene.* 2003; 116: 103-125. doi: 10.1002/ajmg.b.10005

42. Wang TY, Lee SY, Chen SL, et al. Association between *DRD2*, 5-HTTLPR, and ALDH2 genes and specific personality traits in alcohol- and opiate-dependent patients. *Behav Brain Res.* 2013; 250: 285-292. doi: 10.1016/j.bbr.2013.05.015

43. Pijl H, Ohashi S, Matsuda M, et al. Bromocriptine a novel approach to the treatment of type 2 diabetes. *Diabetes Care*. 2000; 23: 1154-1160. doi: 10.2337/diacare.23.8.1154

44. WeenenJEL, Parlevliet ET, Maechler P, et al. The dopamine receptor D2 agonist bromocriptine inhibits glucose-stimulated insulin secretion by direct activation of α2-adrenergic receptors in beta cells. *Biochemical Pharmacology.* 2010; 79: 1827-1836. doi: 10.1016/j.bcp.2010.01.029

45. Rubi B, Ljubicic S, Pournourmohammadi S, et al. Dopamine D2-like receptors are expressed in pancreatic beta cells and mediate inhibition of insulin secretion. *Journal of Biological Chemistry.* 2005; 280: 36824-36832. doi: 10.1074/jbc.M505560200

46. García-Tornadú I, Ornstein AM, Chamson-Reig A, et al. Disruption of the dopamine D2 receptor impairs insulin secretion and causes glucose intolerance. *Endocrinology.* 2010; 151: 1441-1450. doi: 10.1210/en.2009-0996