

Metabolic Syndrome

Chapter 2

Genetic Variants Associated with Components of Metabolic Syndrome in Children and Adolescents

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Abstract

The prevalence of metabolic syndrome is increasing in children and adolescents worldwide. The presence of its risk factor such as obesity, dyslipidemia, glucose intolerance, and hypertension in childhood increases the risk of developing type 2 diabetes and cardiovascular disease in adulthood. Since there is no a consensus definition of the metabolic syndrome, the prevalence varies according with the criteria selected and the ethnicity of the population.

Environmental and genetic factors are involved in the development of metabolic complications; being genetic factors no modifiable. Through strategies like candidate gene studies and Genomic Wide Association Studies, genetic variants have been discovered in complex diseases. Thus, the aim of this chapter is to recopilate the information about genes and genetic variants that have been evidenced to play a role in metabolic syndrome in different children and adolescents populations.

1. Metabolic Syndrome Diagnosis in Children and Adolescents

There is no consensus to define metabolic syndrome (MS) in children and adolescents [1,2], but there are several classifications derived from metabolic syndrome criteria for adults. The most representative are shown in Table 1. According with Cook et al., De Ferranti et al., Weiss et al., and Cruz et al. criteria, the MS is diagnosed when three or more of the components are present. On the other hand, the International Diabetes Federation (IDF), to define MS, requires central obesity plus at least two of four risk factors; besides to consider the ethnicity.

Table 1: Different diagnosis criteria of Metabolic Syndrome in children and adolescents.

Components	Cook <i>et al.</i> [3]	De Ferranti <i>et al.</i> [4]	Weiss <i>et al.</i> [5]	Cruz <i>et al.</i> [6]	IDF (ages 10-16 years). [7]
Obesity	WC \geq p 90th	WC \geq p 75th	BMI Z score \geq 2.0	WC \geq p 90th	WC \geq p 90th
Glucose (mg/dL)	\geq 110	\geq 110	Glycaemia at OGTT of 140-200	\geq 140 (impaired glucose tolerance 2 h)	\geq 100 or T2D
HDL-c (mg/dL)	\leq 40	< 50	< p 5th	\leq p 10th	< 40
Triglycerides (mg/dL)	\geq 110	\geq 100	> p 95th	\geq p 90th	> 150
Blood pressure	\geq p 90th	> p 90th	> p 95th	\geq p 90th	SBP >85mmHg Or treatment with anti-hypertensive medication

WC: waist circumference; BMI: body mass index; OGTT: oral glucose tolerance test; T2D: type 2 diabetes; HDL-c: high density lipoprotein cholesterol; SBP: systolic blood pressure; DBP: diastolic blood pressure; p: percentile.

Despite the differences between criteria, mainly those related with cut-off points for parameters, there are common important risk factors like obesity, impaired glucose metabolism, dyslipidemia and high blood pressure that are consistent with MS diagnosis. Basically, MS has been classified as a group of metabolically interrelated pathophysiologic cardiovascular risk factors that have origins in complex disorders and are influenced by environment and genetics [8]. Candidate gene studies and Genomic Wide Association Studies (GWAS) are strategies to discover genetic variants associated with several complex diseases. However these approaches in children and adolescents are limited and scarce. The main limit is the sample size, and the MS definition used for its diagnosis [9]. In this chapter, studies with different definition criteria of MS are addressed and divided into their main components.

2. Genes Related with Obesity and its Role in Metabolic Syndrome

There is evidence that obesity, inflammation and insulin resistance converge to increased risk of MS. Through the development of bioinformatic tools, genes and metabolic pathways associated with the risk of MS have been identified, mainly with the immune and inflammatory response [10]. The accumulation of body fat characteristic of obesity is accompanied by a low intensity inflammatory state characterized by hypertrophy and hyperplasia of adipose tissue, recruitment of macrophages, and increased production of adipokines, such as Interleukin 6 (IL6), Tumor Necrosis Factor alpha (TNF-a), Interleukin 1 beta (IL- 1b), and leptin processes

that could be activated during childhood [11]. The *IL6R* gene encodes for a receptor of IL6 and its variant rs8192284 has been associated with increased abdominal circumference, triglycerides, and low HDL cholesterol (HDL-c) levels in Taiwanese teenage girls with mean age 13.1 and mean BMI 20.5 Kg/m² [12]. This association was replicated in Spanish adolescents with mean age 14.5 and mean BMI Z-score 1.05. The polymorphism rs1800795 in *IL6* gene, although not directly associated with any component of MS, has an influence on the percentage of body fat-glycaemia in fasting [13]. In a study with Chilean obese children the variants in genes *IL6* and *IL6R* are not associated with an increased risk of MS or with any of its traits. However, certain haplotypes, of low frequency, conformed by polymorphisms in *IL18* are associated with normal levels of triglycerides (≤ 110 mg/dL) and abnormally low levels of HDL-c (<40 mg/dL) [14].

SOCS is a family of intracellular proteins that negatively regulates cytokine signaling by interacting with cytokine receptors and signaling proteins. *SOCS* genes, especially *SOCS3*, display tissue-specific function and are expressed in many tissues and immune regulator cells. Of these, *SOCS1* and *SOCS3*, expressed in beta cells, regulate the Interferon gamma (IFN- γ) signaling pathway [15]. In a study with Turkish morbidly obese and obese children it was found that the frequency of A allele carrier at rs2280148 SNP locus in *SOCS3* was significantly higher in morbidly obese than in obese group, suggesting that A allele may be a risk factor or a marker for morbid obesity [16].

Another molecules that play a critical role in metabolic and inflammatory pathways are the fatty acid binding proteins (FABPs), which serve as intracellular chaperones for lipid moieties [17]. Adipocyte FABP, also known as FABP4, was detected in mature adipocytes, and plays roles in hyperlipidemia, thermogenesis and T2D when obesity is present [18]. So that, FABP4 has been proposed as a bridge between inflammatory processes and other biological pathways related to the metabolic syndrome.

In a study in 309 American children aged 5 to 7 years, researchers found that obese children exhibit higher circulating levels of the pro-atherogenic and pro-inflammatory FABP4 and the rs1054135 polymorphism in *FABP4* gene was significantly more prevalent among obese children [19].

Perilipins are proteins localized at the surface of the lipid droplet in adipocytes, steroid-producing cells, and ruptured atherosclerotic plaques, and they play a key role in the cellular regulation of triglyceride deposition and mobilization [20]. In humans, the perilipin gene (*PLIN*) has been localized to chromosomal location 15q26.1 [21], within a region previously linked to obesity, hypertriglyceridemia, and diabetes [22, 23]. In Brazilian obese children and adolescents aged 7 to 14 years, researchers found that carriers of variant A of *PLIN4* 11482 G \rightarrow A may be at higher risk for MS, whereas being carrier of variant T of *PLIN6* 14995 A \rightarrow T

predicts a better weight loss response to a multidisciplinary behavioral intervention [24].

The matrix metalloproteinases (MMPs) are involved in the development of adipose tissue, modulating adipogenesis and extracellular matrix remodeling [25]. MMP-2 is a type IV collagenase, also known as gelatinase that degrades matrix and non-matrix proteins, particularly basement membrane constituents [26]. It is unknown if MMP-2 plasma concentrations increase whether adipose tissue expands, but dyslipidemia [27], diabetes [28], and metabolic syndrome [29] may affect MMP-2 levels. In a study in Brazilian children and adolescents, researchers found that blood pressure affects the circulating MMP-2 concentrations, and that the CC genotype for the C -1306T polymorphism, in the promoter region of *MMP-2* gene, was more common in controls and obese subjects with higher MMP-2 concentrations, whereas the CT genotype and the T allele for the C-735T polymorphism, in the promoter region of *MMP-2* gene, are less common in obese children than in controls [30].

Another interesting genes are those involved with energy metabolism, such as uncoupling proteins (UCPs) [31]. These proteins participate in the energy balance regulation, cold- and diet-induced thermogenesis, and decreasing the production of reactive oxygen species by mitochondria, all these functions are mechanisms associated with the pathogenesis of obesity and/or T2D [32]. There are several studies in which UCPs were examined as candidate genes for obesity and associated with obesity disturbances such as hypertension, dyslipidemia, and insulin resistance. For example; in Turkish obese children and adolescents, the GG genotype of the *UCP1-3826 A/G* polymorphism was associated with obesity and low HDL-c concentrations, also GG homozygous genotype carriers showed a twofold higher risk of developing obesity than non-carriers. In the same study *UCP2* Exon 8 deletion/insertion (del/ins) polymorphisms were analyzed, and the homozygotes of del/del in *UCP2* gen linked with high body mass index (BMI), likewise del allele was associated with low HDL-c [33]. In Spanish children, obesity was correlated with del allele [34] and in a study which included African American, White and Asian Children, ins variant was related to obesity, and del/ins genotype carriers had markedly higher BMI than those del/del genotype carriers [35].

It is well known that the fundamental disorder related to obesity is insulin resistance (IR), which drives a string of metabolic processes leading to development of proatherogenic lipid profile, type 2 diabetes and high blood pressure [36]. In this regard it is important the study of some genes involved in lipid and glucose metabolism, and blood pressure regulation, as describe below.

3. Genes Related with Hyperlipidemia and its Role with Metabolic Syndrome

The hepatic insulin action impairs when increase the free fatty acid (FFA) flux within the liver, which leads to an increase in the hepatic glucose output, the synthesis of proinflammatory cytokines, the triglycerides (TG) synthesis, the intrahepatic deposition, and the ectopic

lipid accumulation [37]. The excess TG is released with Apo lipoprotein B as very low density lipoprotein (VLDL), the HDL-c secretion diminishes, and the number of relatively cholesterol depleted small dense low density lipoprotein particles increase. These changes in the lipoprotein metabolism are considered the primary cause for MS dyslipidemia [38].

Sterol regulatory element binding factors (SREBFs) are transcription factors playing central roles in the regulation of the carbohydrate and lipid metabolism [39,40]. SREBF-1 and -2 are host genes for miR-33b and miR-33a, respectively [41]. These two microRNAs contribute to the regulation of cholesterol metabolism, β -oxidation of fatty acids, and insulin signaling [42]. In a study in Iranian children and adolescents aged 9 to 19 years were analyzed the polymorphism -1099G>A on *SREBF-1* gene, and was found that HDL-c levels were significantly higher in the MS GG group than in the A allele carrier group. Also, the genotype AA controls (non Metabolic Syndrome) had significantly increased cholesterol and low-density lipoprotein cholesterol (LDL-c) levels than AG genotypes [43].

The gene *PNPLA3* plays an important role in the development of non-alcoholic fatty liver disease (NAFLD), its G allele (148Met, rs738409) is associated with an increased risk of pathological changes of the liver. This variant is upregulated by lipogenic diet and suppressed by favorable x-6:x-3 polyunsaturated fatty acid (PUFA) ratio. Also, high dietary intake of sucrose or fructose have been reported to promote hepatic fat accumulation [44]. In a prospective, observational study in Caucasian population STY-JOBS/EDECTA (STYrian Juvenile Obesity Study/Early Detection of Atherosclerosis, NCT00482924), the researchers found that the *PNPLA3* rs738409 GG genotype is associated already in youths with increased alanine aminotransferase (ALT) plasma concentrations, and is more frequent in obese subjects with Mets in ages 10 to 65 years [45].

Another gene that is important in the regulation of TG and HDL-c levels is the LPL gene. It codes for lipoprotein lipase (LPL). Four *LPL* loss-of-function mutations (p.Asp9Asn, p.Ser45Asn, p.Asn291Ser, and p.Leu365Val) may predispose to atherogenic dyslipidemia in children [46].

The R230C variant in the *ABCA1* gene has been associated with low HDL-cholesterol in several studies in adults; but in Mexican children aged 10 to 13 years, this variant was associated with high triglyceride levels, and with low HDL-c levels [47].

The glucokinase regulator (*GCKR*) gene encodes for a hepatocyte specific inhibitor of the glucose metabolizing enzyme GCK, which plays a key role in maintaining blood glucose homeostasis [48]. GWAS studies have found associations between genetic variants of glucokinase regulator protein (GKRP) and diseases such as T2D, dyslipidemia, and NAFLD in adults [49-51]. The rs780094 single nucleotide polymorphism (SNP) is the most extensively studied genetic variant of the *GCKR* gene. A study in Taiwanese adolescents, mean age 13 years, was

found association between *GCKR* rs780094 polymorphism and low HDL-c levels, also with the incidence of MS [52].

4. Genes Related with Hyperglycemia and its Role with Metabolic Syndrome

Insulin resistance is a pivotal factor in the development of MS and impaired glucose tolerance (IGT). Nowadays, IGT and T2D are much common among obese children and adolescents with a range of incidence between 0.5% to 4% for T2D, and 5% to 25% for IGT [53,54]. It is well known that β -cell dysfunction and hepatic insulin resistance are key defects contributing to the development of T2D. In this way, the study of genes involved in the β -cell function is necessary to understand the development of T2D.

The gene *TCF7L2* encodes for a transcription factor that is a member of the Wnt signaling pathway, an ancient and evolutionarily conserved pathway that regulates crucial aspects of cell fate determination, cell migration, cell polarity, neural patterning and organogenesis during embryonic development, known to be active not only in the β -cells but also in other cell line-ages and glucose-metabolizing tissues, including the liver (55, 56). In a multiethnic cohort of 955 youths, aged 13.3 ± 3.4 years, the rs7903146 variant in the *TCF7L2* gene increases the risk of IGT and T2D in obese adolescents by impairing β -cell function, and hepatic insulin sensitivity [57].

Another gene that was associated with obesity and insulin resistance is the *ENPP1* gene. It encodes to a plasma membrane enzyme termed ENPP1 (ectonucleotide pyrophosphatase phosphodiesterase 1), has been shown to inhibit insulin receptor function by affecting its tyrosine kinase activity in peripheral tissues, including liver, muscle and fat [58]. The rs997509T allele in *ENPP1* can predispose obese children to MS and IGT and might drive the association between the *ENPP1* 121Q allele and insulin resistance [59].

PON 1 is a plasma enzyme that protects low-density lipoproteins (LDL-c) and high-density lipoproteins (HDL-c) against peroxidation [60], also stimulates β -cell insulin secretion and increases the expression of the transport of glucose GLUT4. Therefore this enzyme could be involved in the onset and development of metabolic syndrome [61]. In a study in Mexican children aged 6 to 12 years, the rs662 SNP in *PON 1* gene was associated with insulin resistance and could be considered as a risk marker for insulin resistance [62].

The gene *PCSK1* encodes for a protein convertase 1/3 (PC1/3), this enzyme is an endoprotease that processes many prohormones expressed in endocrine and neuronal cells. Variants in *PCSK1* gene have been associated with obesity risk, body mass index variation, birth weight association with body mass index, and proinsulin levels in several populations [63-65]. In a study in obese German children and adolescents, mean age 11.53 ± 3.36 years, selected for elevated proinsulin levels and/or impaired glucose tolerance, researchers found eight known

variants and two novel heterozygous variants (c.1095 p1G > A and p.S24C) by sequencing the *PCSK1* gene. They identified two rare novel PCSK1 variants of c.1095 p1G>A that caused complete loss of protein function. Also, they confirm rs6232 and rs6234 in *PCSK1* as polygenic risk variants for obesity, and their association with BMI, and rs725522 variant with insulin metabolism [66].

The *GIPR* gene encodes for the gastric inhibitory polypeptide receptor, its ligand is a 42 amino acid incretin released from endocrine K cells of duodenum and the small intestine after oral glucose uptake [67]. Different association studies of single nucleotide polymorphisms (SNPs) in the *GIPR* have been reported and were found in association with obese phenotypes in adults [68]. The role of *GIPR* SNPs on the glucose and insulin homeostasis was recently shown by identifying a variant associated with lower 2 hours insulin levels in non-diabetic patients [69]. In a study in 2280 German children and adolescents, aged 2.5 to 18 years, was observed the association of the exotic SNP rs1800437 and the elevated homeostasis model assessment-estimated insulin resistance (HOMA-IR) values. In addition this variant was in strong linkage disequilibrium with another *GIPR* variant (rs1800437) showing an effect on insulin secretion [70].

5. Genes Related with Hypertension and its Role with Metabolic Syndrome

The prevalence of hypertension and obesity has been increasing worldwide. It is well known that childhood hypertension and obesity predispose to adult hypertension and obesity [71]. The gene *CHCHD5* encodes for a coiled-coil-helix-coiled-coil-helix domain containing 5 (*CHCHD5*), and is a mitochondrial protein which affects mitochondrial oxygen consumption [72]. There is evidence that the SNP rs3748024 in *CHCHD5* was associated with hypertension in Taiwanese adults. Based on this findings, researchers found in Chinese children and adolescents aged 6 to 18 years that the SNP rs3748024 in *CHCHD5* was also associated with systolic blood pressure, BMI, and obesity [73].

The endothelial nitric oxide synthase (eNOS) produce the nitric oxide (NO) and its function is very important to maintain vascular homeostasis, to prevent platelet and leukocyte adhesion, and to inhibit vascular smooth muscle cell migration and proliferation [74]. Deletion in *eNOS* gene promotes hypertension and is associated with other cardiovascular risk factors, frequently found in people with MS, such as insulin resistance, dyslipidemia, hyperuricemia, and increased fibrinogen and leptin levels [75]. In a study in Brazilian obese children and adolescents with MS, the T786C variant in the promoter region of the *eNOS* gene was evaluated, and found that the 786CC genotype show significant association with MS in children and adolescents [76].

The renin-angiotensin-aldosterone system (RAAS) is a major regulator of arterial pressure and as an important endocrine and paracrine system plays a key role in the pathogenesis

of essential hypertension [77]. The angiotensin converting enzyme (ACE) plays an important role in this axis by hydrolyzing angiotensin I into angiotensin II, which has vasoconstriction action. ACE is not only a membrane bound enzyme on the surface of the vascular endothelial cells, but it also circulates in blood plasma [78]. The variant insertion/deletion (I/D) of 287 base pairs in *ACE* gene is associated with the concentration of the circulating enzyme. Individuals homozygous for the D allele have higher tissue and plasma ACE concentrations than heterozygotes and II homozygotes do [79]. In Lithuanian children and adolescents aged 12 to 15 years, researchers found that *ACE* ID and DD genotypes were associated with increased risk for the development of high blood pressure, especially in boys [80].

The genes encoding polyproteins or transport protein are among the main genetic determinants of dyslipidemia. Variants in Apo lipoprotein genes could induce atherogenic changes in the plasma lipid spectrum [81]. In a study in Russian adolescents aged 12 to 18 years, researchers studied interactions between the lipids transporting system genes with essential arterial hypertension (EAH), and with dyslipidemia concomitant with EAH. They found that the maximum contribution to gene-gene interactions entropy was made by allelic polymorphisms *ApoA1* (-75A) and *ApoE* (ϵ 4) and (in the comorbid pathology group) for *ApoE* (ϵ 4) + *ApoB* (Del) [82].

Another gen-gen interaction study was held in Chinese children population, aged 6 to 18 years. Researchers evaluated six hypertension-associated SNPs: *ATP2B1* rs17249754, *CSK* rs1378942, *MTHFR* rs1801133, *CYP17A1* rs1004467, *STK39* rs3754777, and *FGF5* rs16998073. Among the six SNPs, three were associated with obesity risk: *CSK* rs1378942, *MTHFR* rs1801133, and *FGF5* rs16998073. After multiple testing corrections only *ATP2B1* rs17249754 significantly predicted hypertension risk [83].

6. Conclusion

The metabolic syndrome is a clustering of risk factors for cardiovascular disease with complex interactions among its components. Although, studying separately each component of MS can be a very simplistic way of dealing with its etiology, this could allow get closer to the knowledge of this condition. There are many publications of association studies in adult population, in which genetic variants have been related with MS, but the studies about MS in children and adolescents are lacking, Table 2 summarizes the genes that have been associated with MS or any of its components. Most of the published information shows that obesity at early ages plays a key role in the development of other components of MS such as hyperlipidemia, hyperglycemia and hypertension. In addition, several of the genetic variants, mentioned above, were associated with obesity and other risk factors of MS. The comprehension of the MS genetics in children and adolescent population will address more efficiently the treatment of metabolic disorders and prevent de onset of cardiovascular disease and type 2 diabetes.

Table 2: Summary of genes associated with metabolic syndrome or metabolic risk factors.

MS or metabolic risk factor	Gene	Genetic variant	Odds Ratio (95% Confidence Interval)	P	Study population	Age
WC Increased Low HDL-c MS	<i>IL6R</i>	rs192284	1.54 (1.01 to 2.34) 1.49 (1.01 to 2.18) 2.19 (1.15 to 4.51)	< 0.05 < 0.05 < 0.05	Taiwanese girls	Mean age 13.1 years (12)
Morbid obesity	<i>SOCS3</i>	rs2280148 A allele	0.084 (0.020 to 0.356)	< 0.001	Turkish children	Aged 8 to 16.4 years (16)
Increase Risk of MS	<i>PLIN4</i>	11482 G>A	2.4 (1.1 to 4.9)	< 0.001	Brazilian children and adolescents	Aged 7 to 14 years (24)
Low HDL-c	<i>UCPI</i>	UCPI-3826	2.02 (1.17 to 3.47)	0.010	Turkish obese children and adolescents	Mean age 11 years (33)
Atherogenic dyslipidemia	<i>LPL</i>	rs268	8.238 (1.575 to 43.10)	0.010	Italian children and adolescents	Aged 2 to 18 years (46)
Low HDL-c MS	<i>GCKR</i>	rs780094	1.64 (1.07 to 2.53) 2.79 (1.09 to 7.11)	< 0.05	Taiwanese adolescents	Mean age 13 years (52)
Increase Risk of IGT and T2D	<i>TCF7L2</i>	rs7903146	2.224 (1.370 to 3.612)	0.0012	Multiethnic cohort of 955 youths	Mean age 13.3 years (57)
MS IGT	<i>ENPP-1</i>	rs997509T	2.4 (1.3 to 4.3) 4.7 (1.9 to 11.4)	< 0.05	Caucasian obese children and adolescents	Mean age 11.5 years (59)
Insulin resistance (HOMA-IR)	<i>PONI Q</i>	rs662	4.68 (1.23-17.8)	0.016	Mexican children	Aged 6 to 12 years (62)
Obesity	<i>PCSK1</i>	rs6232 rs6234	1.39 (1.00 to 1.93) 1.19 (1.01 to 1.42)	0.022 0.039	German children and adolescents	Mean age 11.53 years (66)
SBP increased BMI increased Obesity	<i>CHCHD5</i>	rs3748024	-1.260 (-1.996 to -0.524) -0.286 (-0.551 to -0.021) 0.828 (0.723 to 0.949)	0.005 0.043 0.018	Chinese children and adolescents	Aged 6 to 18 years (73)
MS	<i>eNOS</i>	T786C	3.27 (1.81 to 9.07)	< 0.05	Brazilian obese children and adolescents	Mean age 10.5 years (76)
High blood pressure	<i>ACE</i>	I/D	2.05 (1.19 to 3.53)	0.01	Lithuanian obese children and adolescents	Aged 12 to 15 years (80)
Obesity risk	CSK MTHFR FGF5	rs1378942 rs1801133 rs16998073	1.2 (1.01 to 1.43) 1.19 (1.05 to 1.34) 1.14(1.0 to 1.29)	0.042 0.006 0.047	Chinese children	Aged 6 to 18 years (83)

MS: Metabolic Syndrome; HDL-c: High Density Lipoprotein cholesterol; IGT: Impaired Glucose Tolerance; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; T2D: Type 2 Diabetes; SBP: Systolic Blood Pressure.

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8. References

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