SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
BIOMARKER RESEARCH
METABOLIC SYNDROME
METABOLIC DISORDERS
GENETICS RESEARCH

Received
4 February 2013

Accepted
4 March 2013

Published
20 March 2013

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A polymorphism of *HMGA1* is associated with increased risk of metabolic syndrome and related components

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The metabolic syndrome (MetS) is a common disorder, where systemic insulin-resistance is associated with increased risk for type 2 diabetes (T2D) and cardiovascular disease. Identifying genetic traits influencing risk and progression of MetS is important. We and others previously reported a functional *HMGA1* gene variant, rs146052672, predisposing to T2D. Here we investigated the association of rs146052672 variant with MetS and related components. In a case-control study from Italy and Turkey, increased risk of MetS was seen among carriers of the *HMGA1* variant. In the larger Italian cohort, this variant positively correlated with BMI, hyperglycemia and insulin-resistance, and negatively correlated with serum HDL-cholesterol. Association between rs146052672 variant and MetS occurred independently of T2D, indicating that *HMGA1* gene defects play a pathogenetic role in MetS and other insulin-resistance-related conditions. Overall, our results indicate that the rs146052672 variant represents an early predictive marker of MetS, as well as a predictive tool for therapy.

The metabolic syndrome (MetS) is a common multicomponent disorder where insulin resistance is associated with an increased risk for type 2 diabetes (T2D), hypertension, dyslipidemia, and cardiovascular disease (CVD)[1,2]. The MetS is also a risk factor for the development of nonalcoholic fatty liver disease[3]. On the basis of the most widely accepted definition from the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III), the clinical diagnosis of MetS is determined by the presence of at least three of the following criteria: abdominal obesity, elevated triglycerides, low high-density lipoprotein-cholesterol (HDL-C), elevated blood pressure (BP), and elevated fasting glucose[4,5].

Current estimates indicate a prevalence of over 30% for the MetS in the United States population[6]. This prevalence, which is increasing worldwide, is a consequence of overnutrition, sedentary lifestyles, and resultant excess adiposity[2]. MetS is associated with excessive and abnormal fat distribution, vascular, hepatic and inflammatory factors, and insulin resistance. This last condition, in particular, seems to play a pivotal role in the pathophysiology of MetS[6-8]. In fact, various individual components of the MetS are significantly associated with insulin resistance[9,10], and cardiovascular risk increases in parallel to insulin resistance both among diabetic and nondiabetic patients[11]. Considerable evidence demonstrates that genetic elements are major contributors to the MetS[12]. Genetic susceptibility of this disorder has been proved by many studies, indicating that the heritability of MetS may vary between 10–30%[13]. Moreover, the large evidence of the clustering of MetS components suggests a common underlying genetic mechanism[14–16], although the precise gene(s) involved still remain largely unknown.

In recent years, a new gene has been associated with insulin resistance and T2D, the high-mobility group AT-hook 1 (*HMGA1*) gene[17–21], which encodes the HMGA1 chromatin binding protein[22]. After binding to AT-rich sequences of DNA, HMGA1 facilitates the assembly and stability of a multicomponent enhancer complex, the "enhanceosome", which drives transcription[22]. HMGA1 is a key regulator of the insulin receptor (*INSR*) gene[17,23]. Defects in HMGA1 cause decreased INSR expression and increased susceptibility to T2D[17–20]. Additionally, the role of HMGA1 in insulin action has been further elucidated by demonstrating that HMGA1 plays a major role in the functional regulation of insulin target genes[24]. Previously, we showed that four functional variants of the *HMGA1* gene were associated with insulin resistance and T2D. The most frequent functional *HMGA1* variant, rs146052672 (http://www.ncbi.nlm.nih.gov/projects/SNP/), also designated IVS5-13insC, was significantly higher in patients with T2D from three populations of white European ancestry[20]. Although not replicated in a heterogeneous French population[25], the *HMGA1* rs146052672 variant was later associated with T2D among Chinese individuals[21], providing further evidence implicating the *HMGA1* locus as one conferring a high cross-race risk of development of T2D.

Given that insulin resistance is a hallmark of both T2D and MetS, we hypothesized that there might be an association between *HMGA1* rs146052672 variant and MetS and that the association might have an impact on the single traits of MetS. Therefore, in the present case-control study, we determined the association of the rs146052672 variant with MetS and its related components in two large Italian and Turkish populations.

Results

The demographic, anthropometric, clinical, and biochemical characteristics of all individuals enrolled in this study are summarized (Table 1). Body mass index (BMI), waist circumference, systolic and diastolic BP, total cholesterol, triglycerides, fasting plasma glucose, and basal insulin were higher in both Italian and Turkish patients with MetS, while HDL-C was higher in the Italian and Turkish control groups. The same trend was observed when these determinations were assessed in the larger Italian control population in those individuals with either one or two components of MetS, as compared to control individuals who had no components of the MetS (Table 2).

Case-control association studies of the *HMGA1* rs146052672 variant in the Italian and Turkish populations. Logistic regression analysis for the effect of *HMGA1* rs146052672 variant on MetS, in both Italian and Turkish populations, were performed (Table 3). Two homozygotes for this mutation were observed among Italian cases, and seven in the Turkish population: four among cases and three among controls (overall Hardy-Weinberg $P = 0.9$). In both populations, the rs146052672 variant was more common in patients with MetS with respect to control subjects. Of the 3,405 Italian patients with MetS, 297 (8.72%) were heterozygous, and 2 (0.06%) homozygous for the rs146052672 variant [minor allele frequency (MAF) was 4.42%]. In contrast, 195 of 5,016 (3.89%) in the total control group were heterozygous carriers (MAF was 1.94%), and no homozygotes were found. For the Italian population, the adjusted Odds Ratio (OR) for age and sex was 2.42 (95% CI, 2.00–2.92), $P < 0.001$ (Table 3). After correction for T2D, the association between *HMGA1* rs146052672 variant and MetS, although to a lesser extent, remained statistically significant [OR 1.75 (95% CI, 1.41–2.18), $P < 0.001$], providing support for a pathogenetic role of *HMGA1* gene defects in MetS and other insulin resistance-related conditions. Adding BMI to the confounder-adjusted model resulted in an increase of the association [OR 2.71 (95% CI, 2.18–3.37), $P < 0.001$] (Table 3). Gender, often suspected as an effect measure modifier, did not modify this association (data not shown).

In view of the larger Italian control population (n = 5,016), a statistically adequate subgroup of control individuals (healthy controls, n = 2,569, statistical power > 0.95) having no components of the MetS, was selected and used in a second case-control analysis of the rs146052672 variant with the Italian patients with MetS

Table 1 | Demographic, anthropometric, clinical and biochemical features of patients with MetS and controls

Italian population	Units	MetS	Control	*P*
Number of subjects	n	3,405	5,016	
Female	n (%)	1,767 (51.9)	2,581 (51.5)	
Age in years	mean ± SD	64.5 ± 3	60.5 ± 13.2	<0.001
BMI in Kg/m^2	mean ± SD	29.8 ± 4.5	24.6 ± 3.4	<0.001
Waist circumference in cm	mean ± SD	102.1 ± 13.9	83.9 ± 12.4	<0.001
Systolic BP in mmHg	mean ± SD	141.5 ± 15.0	128.4 ± 12.6	<0.001
Diastolic BP in mmHg	mean ± SD	83.5 ± 10.8	76.8 ± 10.8	<0.001
Total cholesterol in mg/dL	mean ± SD	188.8 ± 39.3	173.4 ± 31.9	<0.001
HDL-C in mg/dL	mean ± SD	45.8 ± 10.4	53.7 ± 7.0	<0.001
Triglycerides in mg/dL	mean ± SD	153.2 ± 54.3	117.5 ± 29.6	<0.001
FPG in mg/dL	mean ± SD	163.7 ± 43.1	109.7 ± 37.3	<0.001
Basal insulin in μU/mL	mean ± SD	14.5 ± 8.5*	8.4 ± 6.2†	<0.001
Turkish population	**Units**	**MetS**	**Control**	***P***
Number of subjects	n	659	760	
Female	n (%)	476 (72.2)	424 (55.8)	
Age in years	mean ± SD	48.4 ± 12.3	39.6 ± 12.1	<0.001
BMI in Kg/m^2	mean ± SD	30.1 ± 4.5	26.6 ± 4.2	<0.001
Waist circumference in cm	mean ± SD	100.2 ± 10.4	89.1 ± 11.6	<0.001
Systolic BP in mmHg	mean ± SD	141.1 ± 23.9	125.6 ± 20.2	<0.001
Diastolic BP in mmHg	mean ± SD	88.9 ± 12.9	80.7 ± 11.2	<0.001
Total cholesterol in mg/dL	mean ± SD	194.0 ± 41.4	170.4 ± 36.7	<0.001
HDL-C in mg/dL	mean ± SD	37.3 ± 9.2	47.3 ± 12.1	<0.001
Triglycerides in mg/dL	mean ± SD	191.5 ± 120.7	86.2 ± 28.4	<0.001
FPG in mg/dL	mean ± SD	106.5 ± 47.3	87.4 ± 8.3	<0.001
Basal insulin in μU/mL	mean ± SD	10.6 ± 6.6	7.2 ± 4.7	<0.001

The 2-tailed standard *t* test was used for comparisons of means; BP, blood pressure; HDL-C, high-density lipoprotein-cholesterol; FPG, fasting plasma glucose; *n = 834; †n = 2,507.

Table 2 | Clinical and biochemical characteristics of the Italian control population

Controls	Units	With no components of MetS	With one or two components of MetS	*P*
Number of subjects	n	2,569	2,447	
Female	n (%)	1,314 (51.1)	1,267 (51.8)	
Age in years	mean ± SD	58.1 ± 14.4	63.0 ± 11.3	<0.001
BMI in Kg/m^2	mean ± SD	23.1 ± 2.1	26.1 ± 3.8	<0.001
Waist circumference in cm	mean ± SD	79.1 ± 8.6	89.0 ± 13.8	<0.001
Systolic BP in mmHg	mean ± SD	124.2 ± 9.3	132.7 ± 14.0	<0.001
Diastolic BP in mmHg	mean ± SD	73.2 ± 7.7	80.7 ± 12.2	<0.001
Total cholesterol in mg/dL	mean ± SD	164.2 ± 22.9	183.1 ± 36.7	<0.001
HDL-C in mg/dL	mean ± SD	54.4 ± 5.9	53.0 ± 8.00	<0.001
Triglycerides in mg/dL	mean ± SD	112.4 ± 27.2	122.9 ± 30.9	<0.001
FPG in mg/dL	mean ± SD	84.1 ± 6.8	136.5 ± 37.5	<0.001
Basal insulin in μU/mL	mean ± SD	6.5 ± 4.6*	11.8 ± 7.1†	<0.001

The 2-tailed standard *t* test was used for comparisons of means; BP, blood pressure; HDL-C, high-density lipoprotein-cholesterol; FPG, fasting plasma glucose; *n = 1,591 subjects; †n = 915 subjects.

(n = 3,405). In this analysis, the frequency of the rs146052672 variant was lower in healthy controls who had no components of the MetS (carriers 2.57%, MAF 1.28%) than in total controls who had two or fewer components of the MetS (carriers 3.89%, MAF 1.94%), and the adjusted ORs were higher, thus further supporting the association between this variant and MetS (Table 3).

Among the 659 Turkish patients with MetS, 68 (10.3%) were heterozygous and 4 (0.6%) were homozygous carriers of the rs146052672 variant (MAF 5.8%). In comparison (Table 3), of the control group of 760, 54 (7.1%) were heterozygous and 3 (0.4%) homozygous carriers of the variant [MAF 3.9%, OR adjusted for age and sex, 1.66 (95% CI, 1.15–2.39)], $P = 0.006$. After correction for T2D, the association between *HMGA1* rs146052672 variant and MetS increased [OR 1.91 (95% CI, 1.30–2.79), $P = 0.001$]. Similar results were obtained following adjustment for BMI [OR 1.91 (95% CI, 1.29–2.84), $P = 0.001$] (Table 3). Thus, in Turkish population, the MAF of the rs146052672 variant for patients with MetS was higher than for both the Italian patients with MetS and Italian total and healthy controls. The difference in MAF between the Turkish patients with MetS and their controls was statistically significant ($P = 0.001$).

Association of the *HMGA1* rs146052672 variant with indices of MetS in Italian individuals. The association of the *HMGA1* rs146052672 variant with anthropometric, clinical and biochemical indices in patients with MetS was further evaluated by multiple linear regression analysis in the larger Italian population, whose sample size was statistically adequate (statistical power > 0.95) to report reliable results (Table 4). The rs146052672 variant was significantly associated with BMI ($P < 0.001$), with higher fasting plasma glucose levels ($P < 0.001$), and lower HDL-C levels ($P < 0.001$), after adjustment for age and gender. No significant association of the rs146052672 variant was observed with other quantitative traits of the MetS in the Italian population (Table 4).

***HMGA1* rs146052672 variant and insulin resistance.** Finally, we assessed the association of this variant with insulin resistance (Table 5). Basal insulin levels and fasting plasma glucose were measured in a subset of Italian individuals (834 cases and 2,507 controls) who were not taking medications affecting glucose tolerance and whose fasting plasma glucose and insulin levels were available. Linear regression analysis, adjusted for age and sex, showed a positive correlation ($P < 0.001$) of this variant with both fasting plasma glucose and insulin levels, resulting in increased insulin resistance ($P < 0.001$) in carrier individuals, as calculated using the updated HOMA2 method (Table 5).

Discussion

In the present case-control study, we investigated the status of the *HMGA1* rs146052672 variant in two separate population cohorts, Italian and Turkish, with MetS as defined using the NCEP ATP III diagnostic criteria. This variant was present in 9–11% of these

Table 3 | *HMGA1* rs146052672 variant in the Italian and Turkish populations

	−/− n (%)	−/C n (%)	C/C n (%)	MAF (%)	OR 1 (95% CI)	P 1	OR 2 (95% CI)	P 2	OR 3 (95% CI)	P 3
Italian population										
MetS	3,106 (91.2)	297 (8.7)	2 (0.06)	4.42						
Controls (total)*	4,821 (96.1)	195 (3.9)	0 (0)	1.94	2.42 (2.00–2.92)	<0.001	1.75 (1.41–2.18)	<0.001	2.71 (2.18–3.37)	<0.001
Controls (healthy)†	2,503 (97.4)	66 (2.6)	0 (0)	1.28	3.75 (2.84–4.96)	<0.001	3.11 (1.35–3.73)	0.002	3.90 (2.62–5.80)	<0.001
Turkish population										
MetS	587 (89.1)	68 (10.3)	4 (0.6)	5.8						
Controls (total)*	703 (92.5)	54 (7.1)	3 (0.4)	3.9	1.66 (1.15–2.39)	0.006	1.91 (1.30–2.79)	0.001	1.91 (1.29–2.84)	0.001

−/−, Wild-type;
−/C, Heterozygous for rs146052672;
C/C, Homozygous for rs146052672;
OR 1, Age and sex were added as covariates in logistic regression analysis;
OR 2, Age, sex and diabetes were added as covariates in logistic regression analysis;
OR 3, Age, sex and BMI were added as covariates in logistic regression analysis;
CI, Confidence interval;
*Controls with two or fewer components of the MetS;
†Controls without any component of MetS.

Table 4 | Effects of the rs146052672 variant on anthropometric, clinical and biochemical traits in the Italian population

Population	Units	Wild-Type	Carrier	*P*
Number of subjects	n	7,927	494	
BMI in Kg/m^2	mean ± SD	26.7 ± 4.7	27.2 ± 4.2	<0.001
Waist circumference in cm	mean ± SD	91.2 ± 15.9	92.1 ± 13.9	0.118
Systolic BP in mmHg	mean ± SD	133.6 ± 15.1	134.6 ± 13.4	0.099
Diastolic BP in mmHg	mean ± SD	79.5 ± 11.4	80.0 ± 9.7	0.197
Total cholesterol in mg/dL	mean ± SD	179.7 ± 36.0	178.1 ± 33.8	0.424
HDL-C in mg/dL	mean ± SD	50.6 ± 9.3	48.9 ± 10.2	<0.001
Triglycerides in mg/dL	mean ± SD	131.8 ± 45.0	133.7 ± 42.9	0.312
FPG in mg/dL	mean ± SD	130.8 ± 48.1	143.6 ± 40.6	<0.001

Quantitative traits were compared using linear regression analysis adjusted for age and gender. All values have been log-transformed to better approximate a normal distribution.
BP, blood pressure; HDL-C, high-density lipoprotein-cholesterol; FPG, fasting plasma glucose.

patients compared with 4–7.5% of controls, likely representing the most significant genetic risk factor found to date for MetS. According to the results of logistic regression analysis, association between the rs146052672 variant and MetS occurred independently of T2D, and this is further confirmed by the observation that the prevalence of rs146052672 variant in the Italian MetS population (8.8%) was considerably higher than that reported previously in Italian patients with T2D (7.2%) who had been selected regardless of the presence of other MetS components[20]. The association of *HMGA1* rs146052672 variant with MetS irrespective of T2D is consistent with the hypothesis that this variant may independently associate with other insulin resistance-related traits. This assumption is well supported by our results of multiple regression analysis from studies with the larger Italian population showing a strong association of the rs146052672 variant with certain clinical and metabolic quantitative traits related to MetS (i.e. higher BMI, lower HDL-C, higher fasting plasma glucose levels, higher HOMA2-IR). In particular, the association of this variant with an increased HOMA2-IR confirms and extends prior observations[20], indicating that genetic defects negatively affecting HMGA1 protein expression and function, as in the case of the functional *HMGA1* variant rs146052672[20], may play a role in the pathogenesis of MetS and other conditions that are related to insulin resistance.

The relationship of the rs146052672 variant with BMI is consistent with previous observations *in vitro* and *in vivo*, indicating a critical role of HMGA proteins in adipose tissue growth and differentiation[26]. In this regard, whereas suppression of HMGA1 protein function increases growth rate and impairs adipocytic differentiation in 3T3-L1 cells[27], transgenic mice carrying a truncated (dominant-negative) *HMGA1* gene are overweight and accumulate abundant ectopic fat depots[28]. Given that obesity and insulin resistance are major independent risk factors for MetS[29], *HMGA1* gene defects, being mechanistically correlated with both of these traits, may thus represent an important link between insulin resistance, obesity and MetS. It has been recently reported that elevated BMI during adolescence constitutes a major risk factor for later obesity-related disorders, such as coronary heart disease[30]. Correlation of the rs146052672 variant with increased BMI is of interest in the light of this observation, given that early identification of subjects with *HMGA1* gene defects could represent an important tool in the prevention of all obesity-related disorders, as well as a predictive tool for therapy.

Another point that is worth mentioning is the association, in this study, between *HMGA1* rs146052672 variant and low HDL-C, a key component of MetS, which is usually considered as an independent predictor of major cardiovascular events[31]. Association, in the Italian population, of the rs146052672 variant with low HDL-C levels is remarkable and consistent with previous observations, indicating that HMGA1 may affect genes involved in cholesterol homeostasis and reverse cholesterol transport[32].

One last observation from the Italian study population is that the relationship between *HMGA1* rs146052672 variant and MetS was strengthened when logistic regression analysis using parameters from an Italian healthy control group (consisting of individuals with no components of the MetS) produced an OR ~50% greater than that of the total controls who had two or fewer components of the MetS. This result substantiates the specific role of the rs146052672 variant as a novel genetic risk factor for MetS and its components, and supports the hypothesis of a common underlying pathogenetic mechanism for a cluster of conditions that tend to co-occur in

Table 5 | Effects of the *HMGA1* rs146052672 variant on insulin resistance

Italian population	Units	Wild-Type	Carrier	*P*
Number of subjects	n	3,169	172	
Basal insulin in μU/mL	mean ± SD	9.8 ± 7.3	12.6 ± 7.9	<0.001
FPG in mg/dL	mean ± SD	104.3 ± 39.2	118.1 ± 42.4	<0.001
Number of subjects with FPG ≥ 100 mg/dL	n (%)	807 (24.5)	83 (48.3)	<0.001
HOMA2-B%*	mean ± SD	94.7 ± 53.3	99.4 ± 66.2	0.561
HOMA2-S%†	mean ± SD	119.1 ± 73.5	82.4 ± 51.7	<0.001
HOMA2-IR‡	mean ± SD	1.3 ± 1.0	1.7 ± 1.0	<0.001
Number of subjects with HOMA2-IR ≥ 1.8	n (%)	761 (24.0)	72 (41.9)	<0.001

Linear regression analysis was used for assessing the effect of the *HMGA1* variant on HOMA-IR, adding age, gender, and BMI as covariates.
2-tailed Fisher exact test was used for comparisons of proportions.
FPG, fasting plasma glucose.
*Homeostatic model assessment method 2 for beta cells function.
†Homeostatic model assessment method 2 for insulin sensitivity.
‡Homeostatic model assessment method 2 for insulin resistance.
All HOMA2 values have been log-transformed to better approximate a normal distribution.

individuals and in populations. This result confirms the importance in defining the appropriate control group in performing case-control studies of insulin resistance-related diseases[20,33].

Genome-wide association studies and other genetic analyses have been performed to identify genes implicated in MetS[34–38]. However, the genetic variants identified so far, only explain a small proportion of the heritability for MetS and it is not clear how these variants affect the susceptibility to this disorder[39]. An association of MetS with single nucleotide polymorphisms in the *APOC3* (OR = 1.57), *APOA5* (OR = 1.26), *TCF7L2* (OR = 1.18), *FTO* (OR = 1.08), and *CETP* (OR = 0.93) genes has been recently reported in a meta-analysis of findings from 85 prior research studies involving 25 genes[38]. Most of these genes were implicated in lipid homeostasis, thus supporting the concept that disturbances in lipid metabolism play important roles in MetS.

Population stratification is considered by many an important potential confounder in case-control studies of gene-disease association in admixed populations. One particular strength of the present work is the same ethnic origin of cases and controls. We attempted to minimize stratification by using a case-control design in which both Italian cases and controls were collected in a genetically homogeneous population (Calabria, Southern Italy), which is a population of comparatively limited genetic diversity[40,41]. Concerning the other population cohort used in this study, detailed analysis of Turkish population structure has recently been published, suggesting that although this population is admixed, it does not have subpopulation stratification[42].

Overall, we believe our observations have important clinical implications. First, the presence of the rs146052672 variant may serve as an early predictive marker of MetS, in particular in those individuals with a family history of the disease. Second, this information may be used in pharmacogenetics to define patient subpopulations with favorable drug responses. Because the *HMGA1* rs146052672 variant defines a specific defect that can directly affect insulin action and function[20], patients with MetS and this variant may respond differently to a specific therapy. Third, patients with MetS, carrying this *HMGA1* variant, may have a different clinical course than other patients with MetS, including differences in the risk to develop T2D and CVD. In this respect, an involvement of HMGA1 in human atherosclerotic plaques has been reported[43].

In conclusion, these data provide compelling evidence that *HMGA1* is an important susceptibility locus that confers high risk of development of MetS and predisposes to unfavorable anthropometric and metabolic traits of this disorder. Further, the data confirm and extend the observation from previous studies[20,21], that variations in the *HMGA1* gene are indeed associated with the risk of insulin-resistant diseases. Although further investigation is required to better define the molecular role of HMGA1 in MetS, the results in the present report also provide an important contribution to the debate on whether MetS is a unique clinical entity or a cluster of individual disorders all predisposing to CVD[29,44–48].

Methods

Study populations. We studied two populations of patients with MetS from different geographical areas, and ethnic backgrounds. The first and largest population consisted of 3,405 consecutive, unrelated Italian patients with MetS attending the University of Catanzaro outpatient clinics and other ambulatory care sites in Calabria, Italy, between 2007 and 2011. MetS was defined using the NCEP ATP III guidelines, and consisted of meeting at least 3 out of 5 of the following criteria: waist circumference $>$ 102 cm in men and $>$ 88 cm in women, HDL-C $<$ 40 mg/dL ($<$ 1.04 mmol/L) in men and $<$ 50 mg/dL ($<$ 1.29 mmol/L) in women, triglycerides $\geq$ 150 mg/dL ($\geq$ 1.7 mmol/L), BP $\geq$ 130/85 mmHg and fasting glucose $\geq$ 110 mg/dL ($\geq$ 6.1 mmol/L) (Supplementary Table S1)[4,5]. Out of the 3,405 patients with MetS, 871 were diabetics and investigated previously[20]. The Italian control group was identified during the same time period from the same University of Catanzaro's outpatient clinics and other health care sites in Calabria as the cases, in part on the basis of available information from individuals not meeting the criteria for MetS[20]. It consisted of a total of 5,016 unrelated subjects, which included individuals with no components of the MetS (n = 2,569), and individuals with one (n = 1,179) or two (n = 1,268) components of the MetS (Supplementary Table S1). The second patient population included 659 cases and 760 controls who were selected from participants of the Turkish Heart Study recruited between 1990 and 2003[49].

Anthropometric, clinical and biochemical measurements. Anthropometric evaluation included BMI and waist circumference. Waist measurements were made with the subject in erect and relaxed position, considering the smallest horizontal girth between the costal margins and the iliac crests at minimal respiration. BP was obtained from three sphygmomanometric BP measurements with the subject in the sitting position starting 10 min after the beginning of the medical visit. Fasting blood samples were collected and biochemical analyses of plasma glucose, triglycerides, total and HDL-C and serum insulin levels were performed in all participants with no caloric intake for at least 8 h. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated with HOMA2 calculator (http://www.dtu.ox.ac.uk/homacalculator/index.php).

Variant analysis. In both populations, genomic DNA from blood cells was used to genotype the *HMGA1* rs146052672 variant in cases and controls. In 2,139 Italian patients with MetS and in 3,613 Italian controls, this DNA was used as a template for the polymerase-chain-reaction (PCR). The PCR product was purified and sequenced in both sense and antisense directions by a 3100 DNA Genetic Analyzer (Applied Biosystems), or by the Macrogen company (Seoul, South Korea). In the remaining Italian subjects (n = 2,669), and in the entire Turkish population, the fluorescence-based TaqMan allelic discrimination technique (Applied Biosystems) was used as described previously[20]. To verify that the TaqMan and direct sequencing techniques were in agreement, the DNA samples from 160 patients with MetS and 140 controls were directly sequenced for the exon 6 and adjacent introns of the *HMGA1* gene (Genbank n. NC_000006.11, http://www.ncbi.nlm.nih.gov), showing complete concordance with the TaqMan technique. Primers and probes are listed in Supplementary Table S2. These studies were approved by local ethics committees, and informed written consent was obtained for all individuals.

Statistical analysis. Sample size and power calculations were determined using the Javastat software (http://www.statpages.org/proppowr.html) based on the results of a preliminary pilot study in the Italian population. The proportion of controls (n = 300) with the *HMGA1* variant was 0.05, whereas the proportion of cases (n = 300) with the *HMGA1* variant was 0.08, resulting in a minimum sample size estimation of 1,818 cases and 1,818 controls to obtain a statistical power of 0.95 (type II error $<$ 0.05), with a 2-sided significance level of $P < 0.05$. The 2-tailed standard *t* test was used for comparisons of means and the 2-tailed Fisher exact test was used for comparisons of proportions. Generally, a significance level of 0.05 was set for a type I error in all analyses. All MetS-related features were analyzed as either dichotomous traits or continuous quantitative traits using regression models. Because of the low frequency of the *HMGA1* variant, only the dominant genetic model was considered. Logistic regression analysis was used to evaluate individual effects of the *HMGA1* variant as possible risk factor, adding age and gender as covariates. Because of the relatively large number of patients with T2D in the Italian population, adjustment was also made for this disease. Odds Ratios (OR) with 95% confidence bounds were further calculated. Linear regression analysis was employed to compare quantitative biochemical traits between cases and controls after adjustment for covariates. Each quantitative trait was tested for normality using the Shapiro-Wilk normality test and, when required, it was log-transformed. All statistical procedures were performed by using the STATA software version 11.0 (Stata Corp.), except for the calculation of Hardy-Weinberg equilibrium deviation for which the Haploview software was used (http://www.broad.mit.edu/mpg/haploview).

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Acknowledgments

This work was supported by Telethon-Italy (grant GGP04245), and MIUR (protocol 2004062059-002 Italy), to A. Brunetti.

Author contributions

E.C., S.T. and A.B. conceived and designed the experiments; E.C., S.T., A.S., C.C., R.V., R.M., M.G., U.H. and F.P. recruited subjects; E.C., S.T., S.I., B.A., A.N., K.P., F.B., V.V. and G.B. performed the experiments; E.C., S.T., M.P., U.H., D.F. and A.B. analyzed the data; S.T., M.P., F.P. and A.B. contributed reagents/materials/analysis tools; E.C., S.T., F.S.B., U.H., V.D., C.R.P., I.D.G., D.F. and A.B. contributed to the writing of the manuscript. All authors discussed the results and commented on the paper.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Chiefari, E. *et al.* A polymorphism of *HMGA1* is associated with increased risk of metabolic syndrome and related components. *Sci. Rep.* **3**, 1491; DOI:10.1038/srep01491 (2013).