Meta-analysis

The Ala allele in the *PPAR-γ2* gene is associated with reduced risk of type 2 diabetes mellitus in Caucasians and improved insulin sensitivity in overweight subjects

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The purpose of the present study was to identify the association of the Pro12Ala polymorphism in the *PPAR-γ2* gene with diabetes, insulinaemia and insulin resistance. A meta-analysis study was carried out based on studies conducted in the last 10 years, using the databases PubMed, ISI Web of Knowledge, High Wire Press and Scielo, and the reference lists of the obtained articles. We included original studies that showed the relationship between the Pro12Ala polymorphism in the *PPAR-γ2* gene and type 2 diabetes mellitus (T2DM), insulinaemia and insulin resistance. Statistical analyses were conducted using the program RevMAn 5.0. The Mantel–Haenszel test was used to estimate the OR and the 95 % CI of the dichotomous variable, while the standardised effect size was used to estimate the average standardised mean difference and 95 % CI of continuous variables. The studies were subgrouped by ethnicity and overweight status. Forty-one studies were analysed, including a global sample of 30 612 subjects. We found a significant association of the Ala allele with the lowest risk of T2DM in Caucasians (OR 0·80; 95 % CI 0·65, 0·98), lower serum insulin (standardised effect size: −0·05; 95 % CI −0·09, −0·00; *P*=0·04), and greater sensitivity to insulin in overweight individuals (homeostasis model assessment of insulin resistance standardised effect size: −0·07; 95 % CI −0·13, −0·01; *P*=0·02). Considering that the Pro12Ala polymorphism in the *PPAR-γ2* gene is one of the factors related to insulin sensitivity, the present study demonstrated a significant effect of the Ala allele on lower development of T2DM in Caucasians and greater sensitivity to insulin in overweight subjects.

Meta-analyses: Human *PPAR-γ2* polymorphism: Type 2 diabetes mellitus: Insulin resistance

PPAR-γ2 is a nuclear receptor transcription factor, and its codifying gene, on chromosome 3p25, is intensively expressed in adipose tissue[1]. PPAR-γ2 regulates lipid metabolism, adipocyte differentiation, proliferation and insulin sensitivity through regulation of the expression of adipocyte-specific developmental genes[2].

Additionally, there is a missense C-to-G change in codon 12 encoding alanine in substitution for proline in the polypeptide sequence. This polymorphism is relatively common, occurring in 20 % of the Caucasian population[3–5], and seems to be responsible for reduced activity of PPAR-γ2[6].

A few studies have identified an association between the Ala allele and improvement in insulin resistance[3,7–9]. Because the polymorphism is very close to the amino-terminal-activated independent binding domain, its activity is increased by insulin through phosphorylation. It seems that, while proline prevents the α-helix, alanine favours it, and this amino acid change can exert a profound effect on the structure and, consequently, the function of this protein[10].

Given the controversy generated by the studies that report an association between the most frequent polymorphism in the *PPAR-γ2* gene and insulin resistance and diabetes, and also considering that insulin resistance is accountable for promoting metabolic alterations that increase cardiovascular risk in subjects, the present study aimed to identify systematically the association of the Pro12Ala polymorphism in the *PPAR-γ2* gene with type 2 diabetes mellitus (T2DM), insulinaemia and insulin resistance in Caucasians and non-Caucasians and based on overweight status.

Methods

Research design

The present investigation was carried out within a limited period of 10 years, from 1998 to June 2008, using the keywords: ('1998'[publication date]:'2008/06'[publication date]) and ((PPARγ2 polymorphism or peroxisome

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proliferator-activated receptor) and ('insulin resistance' or 'insulin sensitivity' or 'diabetes' or 'impaired glucose tolerance')) and refined by 'humans', 'clinical trial', 'editorial', 'letter', 'meta-analysis', 'randomized controlled trial', adult: 19–44 years, middle aged: 45–64 years, middle aged + aged: 45 + years, aged: 65 + years, 80 and over: 80 + years. The search was performed initially on the PubMed website, which resulted in 152 studies. Other databases were accessed to obtain the full-text articles: ISI Web of Knowledge, MEDLINE, SCIELO, High Wire Press and Science Direct. The reference lists of the original research articles and review articles were used to complement the database search by including additional publications that would not show up in the PubMed search.

Selection of studies was performed independently by two interviewers according to the following exclusion criteria: (1) articles written in languages other than English, Spanish and Portuguese; (2) review articles; (3) missing genotype-specific case numbers or measurement data of fasting insulin or homeostasis model assessment of insulin resistance (HOMA-IR) or diabetes; (4) modified results of fasting insulin or HOMA-IR by log or geometric means; (5) missing measurement of deviation; (6) calculation of HOMA-IR by formulas other than the original represented by Matthews et al.[11]; and/or (7) a genotype distribution not in Hardy–Weinberg equilibrium. Thus, four studies were excluded on the basis of the continuous variables, one due to missing deviation data[12], and another as it transformed data into logarithms to normalise their distribution[2]; the final excluded studies[13,14] calculated insulin resistance by formulas other than HOMA-IR[11].

Forty-one studies were selected for analysis, and all relevant data were extracted individually from each study, including first author, year of publication, country, ethnicity, total number of each genotype of Pro12Ala polymorphism, number of cases representing T2DM and impaired glucose tolerance and controls by normal glucose tolerance and mean and standard deviation for age, BMI, fasting insulin and HOMA-IR.

Data analysis

All analyses were performed on the genotypes Pro12Pro (Pro/Pro) and the sum of Pro12Ala with Ala12Ala (X/Ala). To calculate the mean and standard deviation of X/Ala in some studies presenting separate data, the sum of variances within and between genotypes was used. The same formula was used to group impaired glucose tolerance and diabetes.

When necessary, data were transformed from standard error into standard deviation using the specific formula $SD = SE \times \sqrt{n}$. In addition, serum insulin values presented in μIU/ml were converted into pmol/l by the conversion factor 6·945[15].

Data were analysed by RevMan (version 5.0; The Cochrane Collaboration, Copenhagen, Denmark)[16]. The OR of the Ala allele and T2DM association was calculated by a Mantel–Haenszel test[17]. The inverse of variance with standardised mean difference was used to estimate the association of the Ala allele with serum insulin and HOMA-IR. This measure represents the standardised size effect of polymorphic genotypes (X/Ala) in relation to the wild genotype (Pro12Pro) in fasting serum insulin and HOMA-IR levels. In the course

of the analysis, the studies were separated into subgroups to calculate the summary measure in each subgroup and the overall final measure. The subgroups took into account ethnicity, separating Caucasians from non-Caucasians; the subgroups were also divided according to overweight status, separating the studies that showed an average population of normal BMI ($< 25 \, \text{kg/m}^2$) and increased mean BMI ($> 25 \, \text{kg/m}^2$), classifying them as normal weight and overweight, respectively. The lack of data about the ethnicity of the studies' populations led us to consider as Caucasian those who have ancestry and were born in Europe, or the Middle East, or North Africa, or parts of Central Asia, who share certain genetic and physiological characteristics, beyond white skin.

Statistical analysis of serum insulin in the combination of studies in a population group with normal glucose tolerance and another group with impaired glucose tolerance and T2DM was performed in order to verify that the Ala allele influences the concentration of insulin under different conditions of glucose tolerance.

To assess the statistically significant heterogeneity between studies, a χ^2 test with n-1 df was used, where 'n' is number of studies. In the case of significant heterogeneity in the global analysis, a random-effects model was calculated; otherwise, a fixed-effects model was calculated. Inconsistency (I^2) was calculated to verify how much of the difference between studies was caused by heterogeneity, with values lower than 25 % considered low, 50 % considered moderate, and values greater than 75 % considered high inconsistency[18]. A Z test was used to analyse the global effect and the CI. Significance was assumed at $P < 0.05$.

The outcomes on the left axis that cross the scale (1 or 0) indicate that the corresponding amount is smaller in the X/Ala genotype than the Pro/Pro genotype.

Results

Forty-one eligible studies from the past 10 years were included in the meta-analysis, all of them in Hardy–Weinberg equilibrium. Table 1 describes the main features of each study group while Figs. 1–3 describe the OR and 95 % CI of each group (see below). Citations denoted a, b, c or d represent the same study with different populations.

The association of the Ala allele with T2DM (Fig. 1) included twenty-four studies that showed heterogeneity ($P < 0.00001$). In the overall analysis, the Ala allele had a significant protective association (OR 0·79; 95 % CI 0·66, 0·95). Regarding ethnic differences, the Caucasian subgroup showed a significantly lower risk of developing diabetes for the Ala allele ($P = 0.03$); however, this protective association was not observed among non-Caucasians ($P = 0.21$). Both groups proved to be heterogeneous, but this result disappeared when the Caucasian study Soriguer et al.[19] was deleted (heterogeneity $P = 0.10$; OR 0·86, 95 % CI 0·75, 0·98, $P = 0.02$).

Was also calculated the association between the Ala allele and T2DM according to BMI; both subgroups, normal weight (n 3) and overweight (n 16), showed little association (OR 0·40, 95 % CI 0·25, 0·66, $P = 0.0004$ and OR 0·89, 95 % CI 0·80, 0·99, $P = 0.04$, respectively). The overweight subgroup presented heterogeneity ($P = 0.03$), while the normal-weight subgroup was homogeneous ($P = 0.47$). The total n of

Table 1. Characteristics of the included studies*

Study*	Reference	Subjects (n)	Ala allele frequency	Study population	Age (years)		BMI (kg/m²)		Observations
					Mean	SD	Mean	SD	
Andrulionytè (2004)	20	770	0·23	Mixed	54	4	30·8	4·1	STOP-NIDDM (multicentre)
Altshuler (2000)a	27	962	0·25	Caucasian	60	10	27·5	4·6	Case−control, Scandinavia, T2DM + IGT severe v. NGT
Altshuler (2000)b	27	254	0·18	Caucasian	52	8	29·0	4·5	Case−control, Quebec, T2DM v. NGT
Baratta (2003)	39	338	0·12	Caucasian	37	12	27·0	5·9	Non-diabetic, Sicilian
Beamer (1998)a	40	57	0·17	Caucasian	44	11	38·4	6·4	JHU-WMC, non-diabetic, men
Beamer (1998)b	40	112	0·16	Caucasian	43	10	35·4	9·2	JHU-WMC, non-diabetic, women
Beamer (1998)c	40	316	0·22	Caucasian	64	15	26·9	3·4	BLSA, non-diabetic, men
Beamer (1998)d	40	201	0·19	Caucasian	63	17	25·8	5·0	BLSA, non-diabetic, women
Buzzetti (2004)	31	1215	0·17	Caucasian	42	13	32·7	9·2	Non-diabetic, Italian
Clement (2000)a	33	372	0·11	Caucasian	43	11	47·2	7·5	Morbidly obese (T2DM and NGT)
Clement (2000)b	33	697	0·08	Caucasian	55	9	26·4	5·7	T2DM v. NGT
Deeb (1998)a	7	333	0·23	Caucasian	44	14	25·9	3·3	Non-diabetic, middle-aged, Finnish
Deeb (1998)b	7	973	0·29	Caucasian	70	0·6	27·4	5·1	Elderly subjects, Finnish (T2DM and NGT)
Deeb (1998)c	7	299	0·08	Japanese-American	–		–		Nissei, T2DM + IGT v. NGT
Douglas (2001)a	9	220	0·22	Caucasian	70	0·3	27·0	4·0	FUSION, Finnish, non-diabetic, elderly
Douglas (2001)b	9	193	0·19	Caucasian	61	7	28·4	4·5	FUSION, Finnish, non-diabetic
Douglas (2001)c	9	522	0·15	Caucasian	63	7	30·0	4·8	FUSION, Finnish, diabetic
Ek (2001)a	3	616	0·25	Caucasian	70		25·6	3·1	Swedish men, NGT
Ek (2001)b	3	364	0·26	Caucasian	25	3	23·4	3·5	Healthy, Danish
Ek (2001)c	3	1396	0·24	Caucasian	–		–		Copenhagen, T2DM (n 654) v. NGT (n 742)
Ek (2001)d	3	841	0·25	Caucasian	70		–		Swedish men, NGT (n 616), T2DM + IGT (n 225)
Eurlings (2003)a	4	79	0·28	Caucasian	50	10	27·2	3·0	Cases (T2DM), FCHL, Dutch
Eurlings (2003)b	4	124	0·25	Caucasian	51	11	25·3	3·9	Controls (NGT), FCHL, Dutch
Frederiksen (2002)	41	2245	0·26	Caucasian	40−70		25·8	4·1	Non-diabetic
Hara (2000)a	8	541	0·08	Japanese	69	5	23·8	3·1	Non-diabetic
Hara (2000)b	8	415	0·04	Japanese	61	10	23·5	4·0	T2DM
Hegele (2000)	2	290	0·18	Caucasian	35	14	29·2	5·5	Women, Canadian, T2DM + IGT v. NGT
Jaziri (2006)	42	229	0·08	Caucasian	30−64		–		DESIR, French
Lindi (2002)	43	490	0·31	Caucasian	55	8	31·2	4·6	DPS, IGT, Finnish
Lindi (2003)a	44	150	0·20	Caucasian	49	7	26·5	2·9	KANWU, non-diabetic (NGT + IGT)
Lindi (2003)b	44	72	0·29	Caucasian	49	7	26·9	3·0	KANWU, supplementation n-3 (NGT + IGT)
Kao (2003)	12	1441	0·04	African-American	53	5	29·0	5·8	ARIC, non-diabetic
Mancini (1999)a	10	131	0·13	Caucasian	50	7	27·3	3·5	Italian, T2DM, men
Mancini (1999)b	10	312	0·18	Caucasian	45	6	25·6	3·3	Italian, NGT, men
Meirhaeghe (2000)	45	1009	0·21	Caucasian	51	9	26·6	4·6	WHO-MONICA +T2DM
Memisoglu (2003)	46	1158	0·23	Caucasian	54	7	28·9	5·9	Women
Nicklas (2001)	47	70	0·11	Caucasian	60	7	32·1	4·6	Healthy women, postmenopausal
Niskanen (2003)a	48	119	0·22	Caucasian	54	5	27·1	4·3	NGT
Niskanen (2003)b	48	70	0·20	Caucasian	55	5	30·5	5·2	T2DM
Oh (2000)	24	229	0·08	Asian	48	12	26·0	4·7	Obese and non-obese
Pisabarro (2004)a	28	45	0·13	Caucasian	38	14	31·7	7·1	NGT
Pisabarro (2004)b	28	11	0·55	Caucasian	52	9	39·4	7·5	T2DM + IFG
Radha (2006)a	5	820	0·19	Asian	41	12	23·4	4·6	South-Asians − Chennai (T2DM + NGT)
Radha (2006)b	5	156	0·23	Asian	33	11	23·9	3·6	South-Asians − Dallas (T2DM + NGT)
Radha (2006)c	5	153	0·23	Caucasian	29	7	25·0	5·5	Caucasians − Dallas (T2DM + NGT)
Rhee (2006)	25	253	0·11	Korean	51	6	24·1	2·9	Women
Ringel (1999)	49	924	0·29	Caucasian	–		–		Case−control (T2DM v. NGT)
Rooij (2006)	13	675	0·14	Caucasian	58	1	27·9	1·2	Dutch, T2DM + IGT v. NGT

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Table 1. *Continued*

Study*	Reference	Subjects (n)	Ala allele frequency	Study population	Age (years) Mean	Age (years) SD	BMI (kg/m²) Mean	BMI (kg/m²) SD	Observations
Sánchez (2002)a	50	210	0·17	Caucasian	48	8	27·5	3·8	T2DM + IGT + NGT, obese and non-obese, men
Sánchez (2002)b	50	252	0·17	Caucasian	48	8	28·5	4·9	T2DM + IGT + NGT, obese and non-obese, women
Soriguer (2006)	19	404	0·51	Caucasian	38	13		–	Obese and non-obese, Spanish
Stefanski (2006)	14	216	0·28	Caucasian	64	8	34·2	3·7	T2DM, Poland
Stumvoll (2001)	51	68	0·25	Caucasian	29	7	23·7	3·6	NGT
Swarbrick (2001)a	52	742	0·17	Caucasian	54	12	32·9	2·6	CUDAS and Busselton, obese, T2DM + IGT + IFG + NGT
Swarbrick (2001)b	52	715	0·16	Caucasian	51	13	22·0	1·9	CUDAS and Busselton, non-obese, T2DM + IGT + IFG + NGT
Tai (2004)a	26	3080	0·09	Asian	36	10	23·6	6·7	NGT
Tai (2004)b	26	958	0·09	Asian	50	11	27·0	7·5	T2DM
Tavares (2005)a	38	170	0·11	Brazilian	53	11	25·9	6·5	NGT
Tavares (2005)b	38	207	0·17	Brazilian	54	13	30·6	7·0	T2DM
Temelkova (2004)	32	622	0·28	–	56	7	27·4	4·9	RIAD Study, non-diabetic
Tschritter (2003)a	21	406	0·23	Caucasian	34	18	25·7	5·3	NGT, German
Tschritter (2003)b	21	54	0·24	Caucasian	42	11	28·5	7·9	IGT, German
Valve (1999)	34	141	0·24	Caucasian	43	7	34·8	3·8	Obese, non-diabetic, Finnish
Vänttinen (2005)a	53	72	0·29	Caucasian	29	8	23·0	2·0	Non-obese, non-diabetic, Finnish
Vänttinen (2005)b	53	52	0·27	Caucasian	35	11	32·3	3·9	Obese, non-diabetic, Finnish
Yamamoto (2002)	22	81	0·05	Japanese	43	7	24·7	2·7	Men, hypertensive, non-diabetic
Yamamoto (2002)a	23	478	0·05	Japanese	48	9	23·2	2·7	Men, non-diabetic
Yamamoto (2002)b	23	117	0·07	Japanese	46	8	20·7	2·9	Women, non-diabetic

STOP-NIDDM, Study to Prevent Non Insulin Dependent Diabetes Mellitus; T2DM, type 2 diabetes mellitus; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; JHU-WMC, Johns Hopkins University Weight Management Center; BLSA, Baltimore Longitudinal Study on Age; FUSION, Finland-United States Investigation of Non-Dependent Diabetes Mellitus Genetics Study; FCHL, Familial Combined Hyperlipidaemia; DESIR, Data from an Epidemiological Study on Insulin Resistance Syndrome; DPS, Finnish Diabetes Prevention Study; KANWU, a multi-centre study with five participants (Kuopio/Finland, Aarhus/Denmark, Naples/Italy, Wollongong/Australia, Uppsala/Switzerland); ARIC, Atherosclerosis Risk in Communities; WHO-MONICA, Multinational MONItoring of trends and determinants of Cardiovascular diseases; IFG, impaired fasting glucose; CUDAS, Carotid Ultrasound Disease Assessment Study; Busselton, Busselton Population Health Survey; RIAD, Risk factors in Impaired glucose tolerance for Atherosclerosis and Diabetes.

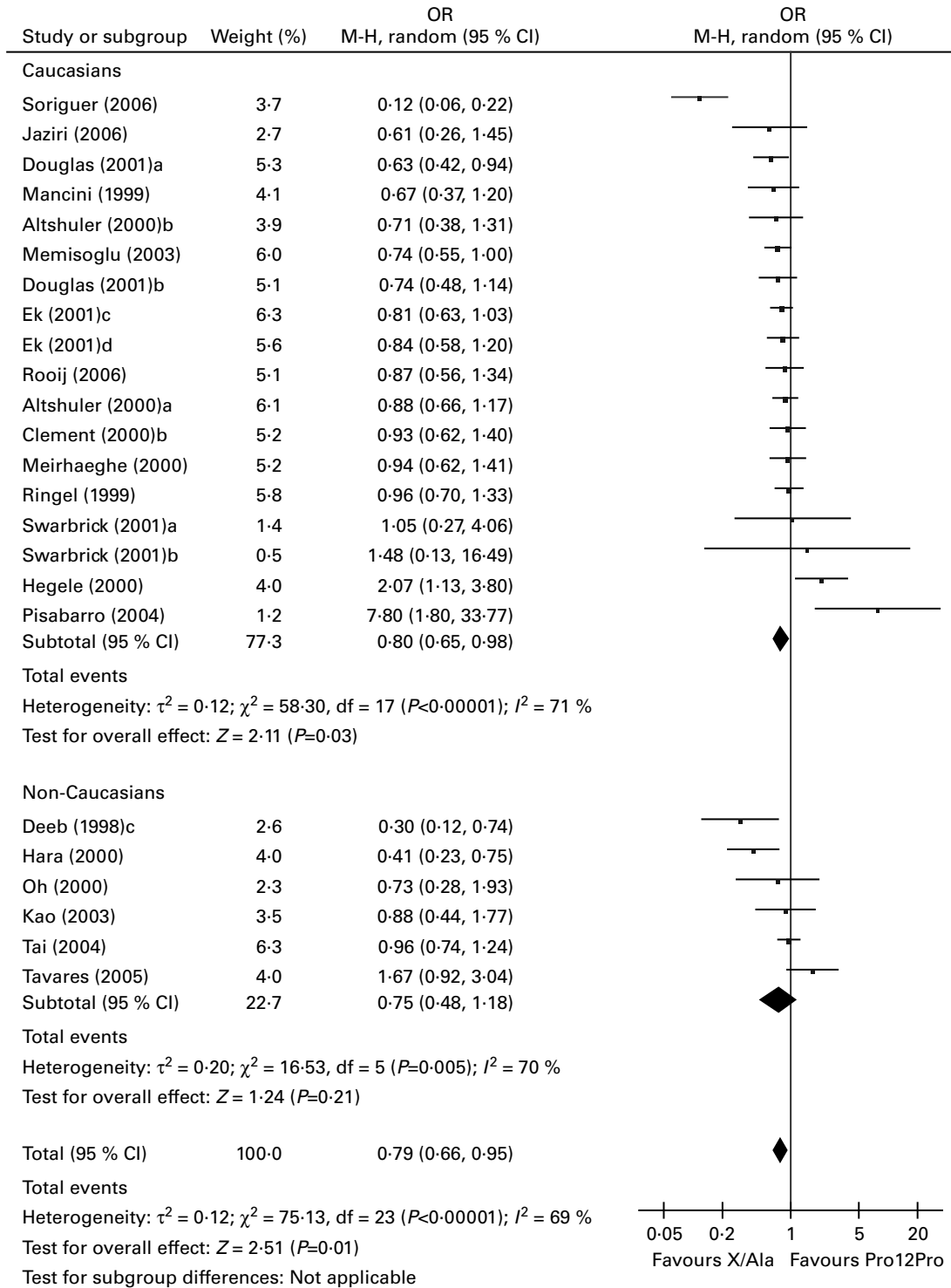* The same study with different populations is shown by a, b, c or d.

Fig. 1. Type 2 diabetes mellitus in eligible studies (sum of Pro12Ala and Ala12Ala (X/Ala) v. Pro12Pro). Estimated standardised effect sizes and CI are given for the single studies and for global comparison. A negative standardised effect indicates that the corresponding frequency is smaller in X/Ala than in Pro12Pro. M-H, Mantel–Haenszel. The reference numbers for the studies can be found in Table 1. The same study with different populations is shown by a, b, c or d.

this analysis was smaller (n 19) due to failure to provide the average BMI in some studies, so it was not possible to include these studies in one of the subgroups.

The association of serum insulin with the Ala allele showed that insulin concentration is lower in individuals of this allele compared with the wild genotype, but this relationship was not significant (OR −0·04; 95 % CI −0·09, 0·01; P=0·09); the sample proved to be heterogeneous (P=0·02). The I^2 test was 30 %, indicating moderate inconsistency between studies. Grouping by ethnicity, Caucasians (n 36) and non-Caucasians (n 13), and excluding the multi-centre Andrulionytè et al.[20], produced the following results

Study or subgroup	Weight (%)	Standard mean difference IV, fixed (95 % CI)	Standard mean difference IV, fixed (95 % CI)
Normal weight			
Hara (2000)b	0·5	−0·50 (−1·02, 0·01)	
Vänttinen (2005)a	0·5	−0·45 (−0·96, 0·06)	
Stumvoll (2001)	0·5	−0·30 (−0·85, 0·25)	
Ek (2001)b	2·5	−0·11 (−0·34, 0·13)	
Hara (2000)a	1·5	−0·04 (−0·35, 0·26)	
Tai (2004)a	9·2	−0·04 (−0·16, 0·08)	
Radha (2006)a	4·6	0·00 (−0·17, 0·17)	
Yamamoto (2002)b	0·3	0·00 (−0·72, 0·72)	
Radha (2006)b	1·0	0·09 (−0·28, 0·46)	
Yamamoto (2002)	0·1	0·13 (−0·87, 1·14)	
Rhee (2006)	0·9	0·16 (−0·24, 0·56)	
Yamamoto (2002)a	0·8	0·20 (−0·21, 0·61)	
Subtotal (95 % CI)	22·4	−0·04 (−0·12, 0·04)	

Heterogeneity: $\chi^2 = 9\cdot75$, df = 11 ($P=0\cdot55$); $I^2 = 0$ %
Test for overall effect: $Z = 1\cdot06$ ($P=0\cdot29$)

Study or subgroup	Weight (%)	Standard mean difference IV, fixed (95 % CI)	
Overweight			
Pisabarro (2004)b	0·1	−0·51 (−1·72, 0·71)	
Vänttinen (2005)b	0·5	−0·45 (−0·96, 0·06)	
Tavares (2005)b	1·1	−0·39 (−0·75, −0·03)	
Tavares (2005)a	0·6	−0·34 (−0·82, 0·14)	
Niskaner (2003)b	0·4	−0·33 (−0·92, 0·26)	
Sánchez (2002)b	1·2	−0·33 (−0·66, 0·01)	
Pisabarro (2004)a	0·2	−0·29 (−1·15, 0·57)	
Deeb (1998)a	2·0	−0·27 (−0·53, −0·01)	
Stefanski (2006)	1·6	−0·21 (−0·51, 0·09)	
Mancini (1999)b	1·7	−0·21 (−0·49, 0·08)	
Buzzetti (2004)	6·1	−0·20 (−0·35, −0·05)	
Tschritter (2003)a	2·6	−0·18 (−0·41, 0·06)	
Beamer (1998)d	1·1	−0·16 (−0·51, 0·19)	
Sánchez (2002)a	1·1	−0·16 (−0·52, 0·20)	
Eurlings (2003)a	0·6	−0·14 (−0·63, 0·36)	
Radha (2006)c	1·0	−0·12 (−0·50, 0·26)	
Baratta (2003)	1·3	−0·09 (−0·41, 0·24)	
Beamer (1998)b	0·5	−0·08 (−0·59, 0·42)	
Tai (2004)b	2·8	−0·07 (−0·29, 0·15)	
Lindi (2003)a	1·0	−0·03 (−0·41, 0·34)	
Frederiksen (2002)	15·3	−0·02 (−0·11, 0·07)	
Lindi (2002)	3·8	−0·01 (−0·20, 0·18)	
Ek (2001)a	4·2	−0·01 (−0·19, 0·17)	
Andrulionytè (2004)	4·9	−0·01 (−0·18, 0·16)	
Mancini (1999)a	0·5	0·00 (−0·51, 0·51)	
Eurlings (2003)b	0·8	0·00 (−0·40, 0·41)	
Oh (2000)	0·6	0·03 (−0·45, 0·51)	
Deeb (1998)b	7·1	0·04 (−0·10, 0·18)	
Temelkova (2004)	4·5	0·05 (−0·12, 0·23)	
Clement (2000)a	2·2	0·08 (−0·17, 0·33)	
Nicklas (2001)	0·3	0·11 (−0·59, 0·81)	
Beamer (1998)c	2·0	0·13 (−0·14, 0·39)	
Valve (1999)	0·9	0·22 (−0·17, 0·61)	
Lindi (2003)b	0·5	0·26 (−0·25, 0·77)	
Niskaner (2003)a	0·7	0·29 (−0·14, 0·73)	
Beamer (1998)a	0·3	0·35 (−0·33, 1·04)	
Douglas (2001)	1·5	0·45 (0·15, 0·75)	
Tschritter (2003)b	0·0	1·20 (0·53, 1·86)	
Subtotal (95 % CI)	77·6	−0·04 (−0·09, −0·00)	

Heterogeneity: $\chi^2 = 47\cdot44$, df = 36 ($P=0\cdot10$); $I^2 = 24$ %
Test for overall effect: $Z = 2\cdot04$ ($P=0\cdot04$)

Total (95 % CI)	100·0	−0·04 (−0·08, −0·01)	

Heterogeneity: $\chi^2 = 57\cdot20$, df = 48 ($P=0\cdot17$); $I^2 = 16$ %
Test for overall effect: $Z = 2\cdot30$ ($P=0\cdot02$)
Test for subgroup differences: $\chi^2 = 0\cdot00$, df = 1 ($P=0\cdot97$), $I^2 = 0$ %

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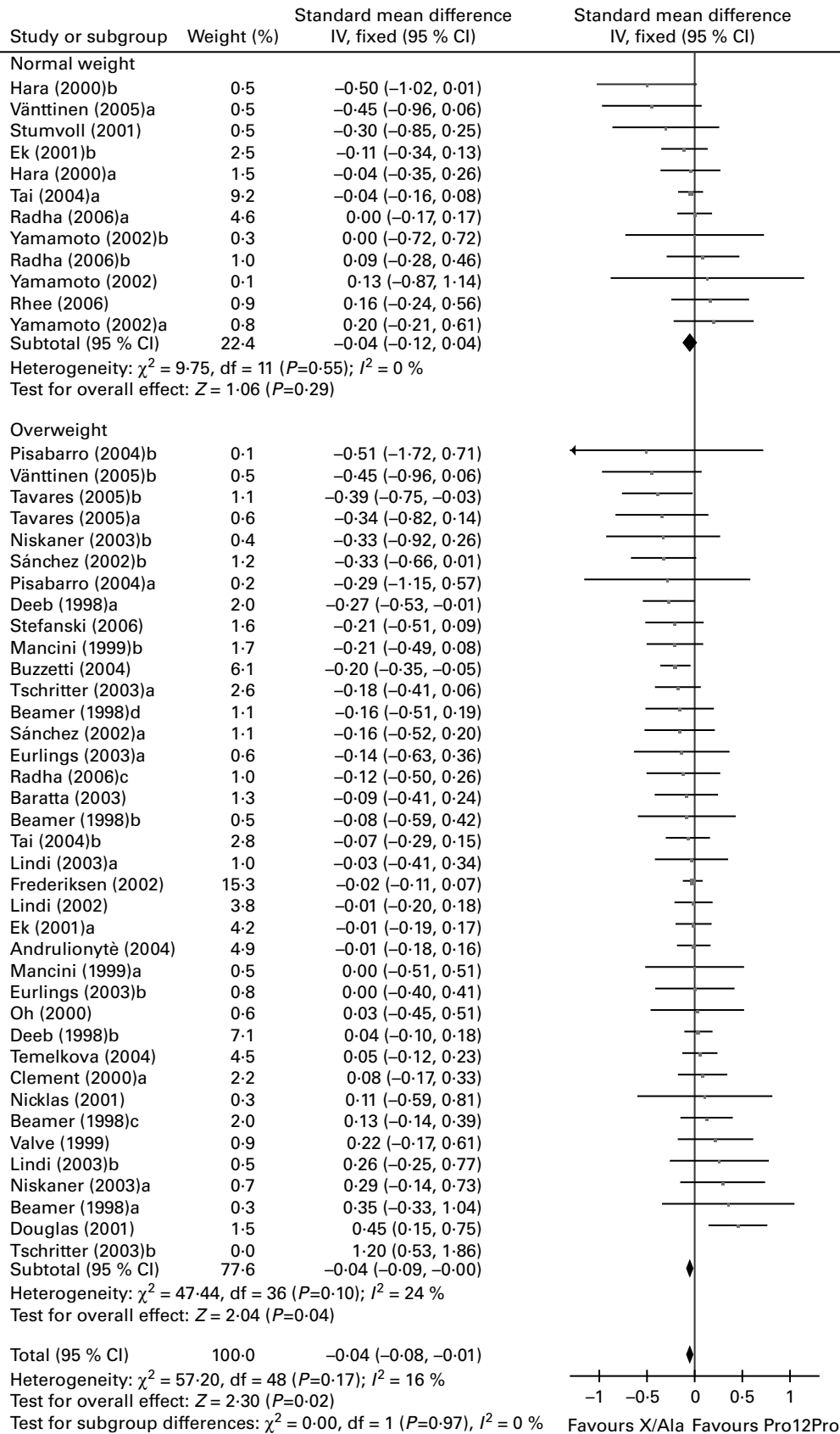Favours X/Ala   Favours Pro12Pro

Fig. 2. Serum insulin in eligible studies (sum of Pro12Ala and Ala12Ala (X/Ala) v. Pro12Pro). Estimated standardised effect sizes and CI are given for the single studies and for global comparison. A lower insulin concentration was significant for Ala allele carriers excluding Tschritter et al.[(21b)] ($P=0\cdot02$). IV, insulin values. The reference numbers for the studies can be found in Table 1. The same study with different populations is shown by a, b or c.
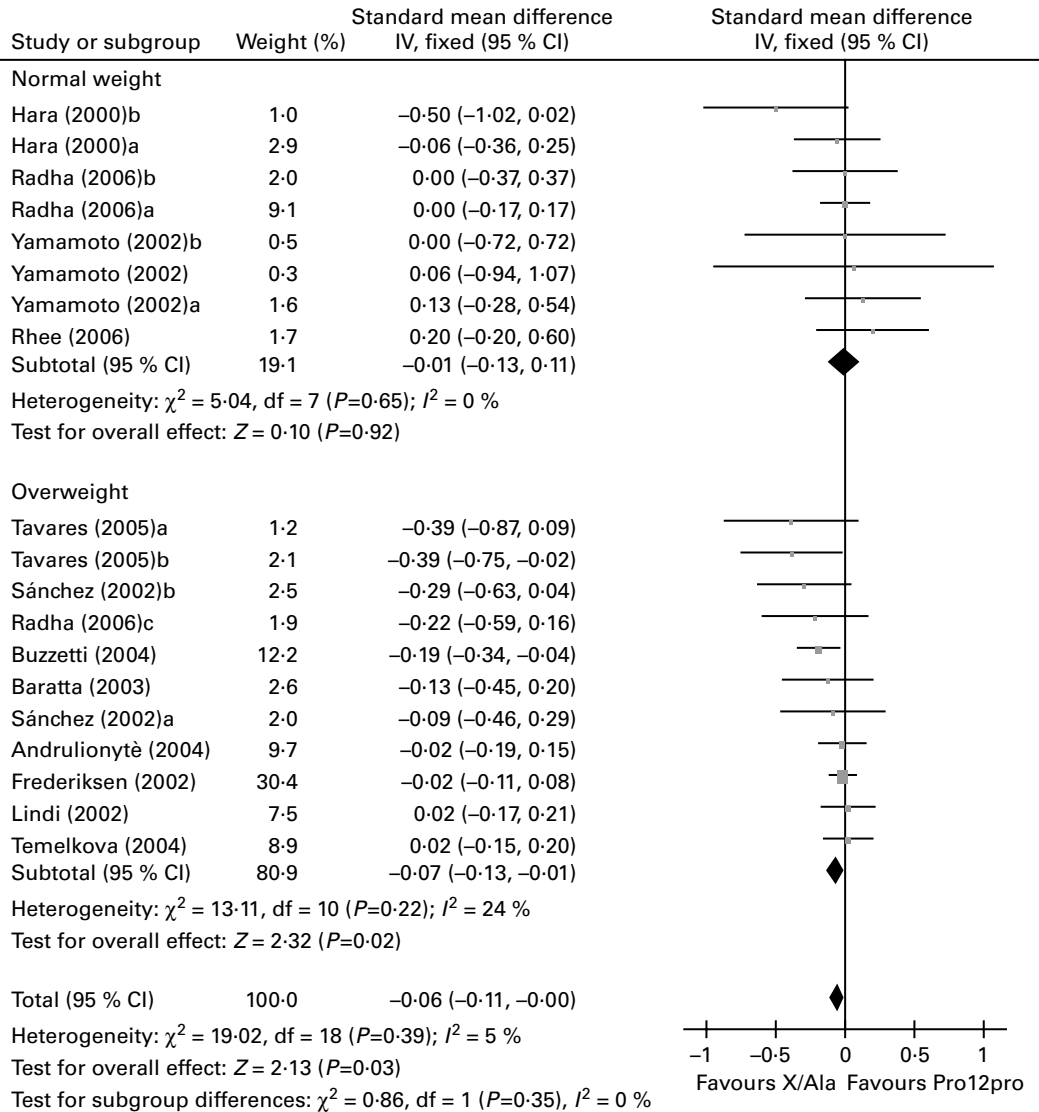
Study or subgroup	Weight (%)	Standard mean difference IV, fixed (95 % CI)	Standard mean difference IV, fixed (95 % CI)
Normal weight			
Hara (2000)b	1·0	−0·50 (−1·02, 0·02)	
Hara (2000)a	2·9	−0·06 (−0·36, 0·25)	
Radha (2006)b	2·0	0·00 (−0·37, 0·37)	
Radha (2006)a	9·1	0·00 (−0·17, 0·17)	
Yamamoto (2002)b	0·5	0·00 (−0·72, 0·72)	
Yamamoto (2002)	0·3	0·06 (−0·94, 1·07)	
Yamamoto (2002)a	1·6	0·13 (−0·28, 0·54)	
Rhee (2006)	1·7	0·20 (−0·20, 0·60)	
Subtotal (95 % CI)	19·1	−0·01 (−0·13, 0·11)	

Heterogeneity: $\chi^2 = 5\cdot04$, df = 7 ($P=0\cdot65$); $I^2 = 0$ %
Test for overall effect: $Z = 0\cdot10$ ($P=0\cdot92$)

Overweight			
Tavares (2005)a	1·2	−0·39 (−0·87, 0·09)	
Tavares (2005)b	2·1	−0·39 (−0·75, −0·02)	
Sánchez (2002)b	2·5	−0·29 (−0·63, 0·04)	
Radha (2006)c	1·9	−0·22 (−0·59, 0·16)	
Buzzetti (2004)	12·2	−0·19 (−0·34, −0·04)	
Baratta (2003)	2·6	−0·13 (−0·45, 0·20)	
Sánchez (2002)a	2·0	−0·09 (−0·46, 0·29)	
Andrulionytè (2004)	9·7	−0·02 (−0·19, 0·15)	
Frederiksen (2002)	30·4	−0·02 (−0·11, 0·08)	
Lindi (2002)	7·5	0·02 (−0·17, 0·21)	
Temelkova (2004)	8·9	0·02 (−0·15, 0·20)	
Subtotal (95 % CI)	80·9	−0·07 (−0·13, −0·01)	

Heterogeneity: $\chi^2 = 13\cdot11$, df = 10 ($P=0\cdot22$); $I^2 = 24$ %
Test for overall effect: $Z = 2\cdot32$ ($P=0\cdot02$)

Total (95 % CI)	100·0	−0·06 (−0·11, −0·00)	

Heterogeneity: $\chi^2 = 19\cdot02$, df = 18 ($P=0\cdot39$); $I^2 = 5$ %
Test for overall effect: $Z = 2\cdot13$ ($P=0\cdot03$)
Test for subgroup differences: $\chi^2 = 0\cdot86$, df = 1 ($P=0\cdot35$), $I^2 = 0$ %

Favours X/Ala   Favours Pro12pro

Fig. 3. Homeostasis model assessment for insulin resistance for eligible studies (sum of Pro12Ala and Ala12Ala (X/Ala) v. Pro12Pro). Estimated standardised effect sizes and CI are given for the single studies and for global comparison. A negative standardised effect size indicates that the corresponding quantity is smaller in X/Ala than in Pro12Pro. IV, insulin values. The reference numbers for the studies can be found in Table 1. The same study with different populations is shown by a, b or c.

for Caucasians: OR −0·04 (95 % CI −0·11, 0·02, $P=0\cdot22$); and non-Caucasians: OR −0·05 (95 % CI −0·12, 0·03, $P=0\cdot23$). The subgroup of Caucasians showed heterogeneity ($P=0\cdot01$), with 39 % of the studies contributing to this, according to results of I^2; however, the subgroup of non-Caucasians was homogeneous ($P=0\cdot49$).

As seen in Fig. 2, the study that differed the most was Tschritter et al.[21b], whose sample, after being excluded from the analysis, showed no global heterogeneity ($P=0\cdot17$); the meta-effect size calculation using a fixed-effects model proved to be significant (standardised mean difference: −0·05; 95 % CI −0·09, −0·00; $P=0\cdot02$). Thus, both subgroups, normal weight and overweight, presented themselves as homogeneous, with a negative association between the Ala allele and the concentration of insulin, although the effects were not significant ($P=0\cdot029$ and $P=0\cdot07$, respectively).

When separating the diabetic (n 12) and glucose-tolerant (n 30) groups, if the study of Tschritter et al.[21b] study is removed, analysis of the Ala allele is associated with lower insulin concentrations for the diabetic group (standardised mean difference −0·13, 95 % CI −0·24, −0·02, $P=0\cdot02$; heterogeneity $P=0\cdot53$).

The same protective effect of the Ala allele is observed in the outcome of the association of HOMA-IR with polymorphism (Fig. 3), where the standardised effect size is significantly lower in the Ala allele carriers ($P=0\cdot03$) than in Pro12Pro genotype. The heterogeneity result in this analysis was not significant ($P=0\cdot39$), and the inconsistency test showed very low inconsistency. According to the subdivisions shown in Fig. 3, the overweight subgroup showed the lowest HOMA-IR values associated with the Ala allele ($P=0\cdot02$). However, the test to evaluate the difference between the groups was not significant ($P=0\cdot35$).

In the analysis by ethnicity, both the Caucasian and non-Caucasian subgroups were homogeneous ($P=0.41$ and $P=0.29$, respectively). Despite the association of each ethnic subgroup showing the same trend of the overall result (Caucasians' standardised mean difference -0.06, 95 % CI -0.11, 0.00, $P=0.07$; non-Caucasians' standardised mean difference -0.06, 95 % CI -0.17, 0.05, $P=0.27$), described previously, the associations were not significant.

Discussion

The studies presented in the present meta-analysis showed great variability in their frequency of polymorphism, ranging from 0.04 to 0.55; the samples with the lowest rates included non-Caucasian populations (African[12], Japanese[7c,8a,8b,22,23a,23b], Korean[24,25] and Asian descendants in general[26a]). The lower frequencies of polymorphism coincided with lower average BMI; however, in one population of Asians with a higher mean BMI, the frequency remained low[5a], similar to the results of previous studies[7c,8a,8b,12,22–26a].

Of the selected studies, twenty investigated Caucasian populations, and eleven European populations; only eight Asian populations, a North American and a South American population were included (Table 1). It should be noted that studies with European populations that did not clarify the ethnicity of the individuals included were then considered Caucasians, due to the low mixture of races of these populations. Interest in investigating the influence of the Pro12Ala polymorphism on the development of T2DM and insulin resistance appears to be higher in Caucasian populations, probably because of the greater frequency of this polymorphism among them.

In the present meta-analysis, twenty-four recent studies were included in the analysis of the association between the Ala allele and T2DM (Fig. 1), resulting in a protective effect of this allele to lower the risk of developing diabetes similar to Altshuler et al.[27] (n 11; OR 0.79; $P=0.00007$) and Ek et al.[3] (n 10; OR 0.81; 95 % CI 0.72, 0.91; $P=0.00034$). Furthermore, the risk of T2DM in Ala allele carriers could be shown to differ among ethnic groups, as the OR was lower in Caucasians and there was no significant risk in non-Caucasians (including Asians, African-Americans and South Americans). However, there was an increase in the overall CI and heterogeneity within Caucasian and non-Caucasian subgroups.

Regarding ethnicity, the meta-analysis performed by Ek et al.[3] showed that both Caucasians (n 7) and Asians (n 3) presented a significant negative association between the Ala allele and T2DM. However, there was a statistical difference between the OR of the two ethnic groups because the strength of association was lower for Asians than for Caucasians (Asian OR 0.42, 95 % CI 0.26, 0.67 $v.$ Caucasian OR 0.85, 95 % CI 0.76, 0.96; $P=0.0033$). Nevertheless, Radha et al.[5] found in a study conducted with one Caucasian and two Asian populations that the Ala allele did not protect South Asian populations against T2DM, but did protect the Caucasians. This study found no significant difference between the polymorphism frequency in South Asian diabetics and non-diabetics (20 $v.$ 23 % in the Dallas cohort and 19 $v.$ 19.3 % in the Chennai cohort; $P>0.05$). Thus, both studies

corroborate the present meta-analysis by suggesting that the Ala allele is a protective factor for T2DM in Caucasian populations.

Only four studies[7c,9a,8,19] observed a significantly inverse association between the Ala allele and T2DM (i.e. found that the Ala allele is not associated with T2DM). Two of these analysed Nissei (second-generation Japanese) populations, one living in the Occident and another in Japan; the first[7] showed a strong association between the wild genotype (Pro12Pro) and T2DM (OR 4.35; 95 % CI 1.24, 15.3; $P=0.028$). The other studies were carried out on Caucasians.

Pisabarro et al.[28] reported that Ala allele carriers developed T2DM at a younger age. Regarding sex, the Pro12Ala polymorphism was found to be strongly associated with T2DM in women but not in men[2]. The heterogeneity shown in Fig. 1 demonstrates that there is a high probability that the difference between OR is not due to chance (random error), but rather expresses different effects, probably influenced by age[28], sex[2] or even lipids in the diet[29].

Those with the Ala allele had lower insulin concentrations in the global analysis, but this effect becomes significant when removing Tschritter et al.[21], which used a sample of overweight subjects with impaired glucose tolerance. This result confirms the meta-analysis performed by Tönjes et al.[30], who showed a standardised effect size of 0.168 ($P=0.040$) for Ala12Ala's association with lower insulin concentrations compared with the Pro12Pro genotype. However, they verified no significant serum insulin effect in association with the Ala allele. Their study assessed only non-diabetic samples[30] that still showed homogeneity ($P=0.052$).

Other studies found an association between the Ala allele and the lowest insulin concentration and increased sensitivity to insulin, regardless of sex[7], BMI[27], being non-diabetic[31] and age[32]. Kao et al.[12] showed variations in BMI and fasting insulin depending on Pro12Ala genotype ($P=0.0027$). Two other studies showed lower obesity levels associated with insulin genotype Pro12Pro[33,34], but there was no apparent similarity between the studies that had shifted to the right on the graph of this analysis.

While the Ala allele is associated with a lower risk of developing diabetes, it is interesting to note that even under conditions of abnormal glucose tolerance, this polymorphism was associated with lower concentrations of insulin. This contributes to cardiovascular risk factors because hyperinsulinaemia is involved in several cardiovascular pathophysiological mechanisms[35–37].

The beneficial effect of the Ala allele on sensitivity to insulin observed in the present study covered nineteen samples that analysed mean HOMA-IR, with one of the two samples composed of Brazilian diabetics being of significantly negative association[38]. Using diabetic and non-diabetic samples in the same meta-analysis enriched the present study because it is assumed that despite the different values found for glucose-tolerant individuals, the difference between the wild genotype and Ala allele averages would influence the final result.

The insulin resistance values were significantly lower in the group carrying the Ala allele (Fig. 3), as in Tönjes et al.[30] in the obesity group, although a significant association in the overall HOMA-IR effect was present, highlighting the power of association in this study through homogeneity among the studies selected for this analysis.

The present study has endeavoured to standardise the measures examined in order to minimise bias and heterogeneity between studies; stratification analysis was also performed to better characterise the subgroups.

Final considerations

These results suggest that the Ala allele is protective against T2DM development in Caucasians and not in some of the other populations. However, the mechanism has not been fully elucidated in the literature. It is important to emphasise that sensitivity to insulin is influenced by multiple factors; Pro12Ala polymorphism of the gene *PPAR-γ2* is one of them.

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