

# **Pro12Ala polymorphism of the peroxisome proliferator-activated receptor $\gamma$ 2 (PPAR $\gamma$ 2) gene and type 2 diabetes mellitus**

*Protocol for a HuGE association review*

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## Background

Type II diabetes mellitus (T2DM) currently affects over 150 million people world wide, with a prevalence that varies markedly from population to population. Predictions for the year 2025 estimate that almost 300 million people will suffer from diabetes mellitus and the vast majority of cases will be T2DM. T2DM currently accounts for 90% of diabetes cases. The disease usually develops after the age of 40 with a mean age of diagnosis around 60 and is one of the main causes of blindness, lower limb amputations and renal failure. Due to macrovascular complications, it is also a major risk factor for cardiovascular disease and stroke. These chronic consequences have a high socio-economic cost and put a heavy burden on public health services, the World Health Organisation (WHO) has estimated that the disability-adjusted life years lost for T2DM is 1.1 years (1, 2). Evidence suggests that T2DM is a multifactorial genetic syndrome, determined by several different genes and environmental factors. Genetic susceptibility is indicated by the varying risk across populations (from 5% in white populations to 50% in Pima Indians), twin studies, and excess concordance rates in monozygotic versus dizygotic twins clearly advocate a contribution of genetic factors. Due to the heterogeneity and probably polygenicity of the disease, few genetic variants have been identified to account for a substantial proportion of common T2DM. It is expected that characterising the genetic risk will offer a better understanding of the disease pathology and therefore, more effective therapies. This knowledge may also aid in the identification of the non-genetic factors at play and thus provide targets for cost-effective intervention(3-5).

Since the discovery of loss-of-function mutations in the PPAR $\gamma$  gene (12), it has gained much attention as a candidate gene potentially associated to T2DM. PPAR $\gamma$  is a nuclear transcription factor and plays a major role in the regulation in adipogenesis. It is located on chromosome 3 and is expressed in two isoforms resulting from alternative mRNA splicing (13-15). Evidence of an association between Pro12Ala polymorphism in PPAR $\gamma$ 2 and T2DM was first obtained in 1997 from a study on Japanese-Americans (16). A frequency of the rare Ala allele of 9.3% in the control group versus only 2.2% in patients with T2DM was observed. Characteristic of first publications, a rather optimistic estimate of risk reduction was reported (17, 18), implying a 75% risk reduction. Only one of five subsequent studies replicated this association. In 2000, however, Altshuler *et al.* (19) using a family-based transmission disequilibrium test (TDT) design to control for population stratification, and conducting a meta-analysis using this data, confirmed the association between Pro12Ala and T2DM. Their conclusions suggested that the individual risk of the polymorphism was low at about 1.25, but due to the high prevalence of the Pro12 allele (85% in Caucasians), the population-attributable risk influences 25% of all T2DM. Lohmueller's *et al.* (20) meta-analysis reconfirmed Altshuler's findings and also showed that publication bias was not a likely concern. Neither of these meta-analyses involved a systematic review nor gave any justification for the genetic model used in their analysis.

Pro12Ala has also been implicated in gene-disease associations with obesity, insulin resistance, cardio-vascular disease and some forms of cancer.

Reference	Number of Studies	Number of Participants	Clinical Outcomes	Genetic Model	Allele Specific Odds Ratio (95% CI)
Altshuler, D <i>et al.</i> (2000)	7	3000	IGT and IFG	NA	1.25
Lohmueller, KE <i>et al.</i> (2003)	14	NA	NA	NA	FE = 1.22 (1.08-1.37) RE = 1.21 (1.07-1.37)

(NA, Not available; FE, Fixed effects; RE, Random effects; IGT, impaired glucose tolerance; IFG, impaired fasting glucose)

**Table 1:** All meta-analyses conducted on genetic association studies of Pro12Ala polymorphism of the PPAR $\gamma$ 2 gene with T2DM.

### Objective

To quantify the magnitude of association between Pro12Ala polymorphism of the PPAR $\gamma$ 2 gene and type 2 diabetes mellitus.

### Methods

#### Criteria for considering studies

##### Polymorphisms

Study selection will be limited to those addressing, specifically, the Pro12Ala polymorphism of the PPAR $\gamma$ 2 gene.

##### Clinical outcomes

The clinical outcome of interest is T2DM (as defined by impaired glucose tolerance and impaired fasting glucose).

##### Types of studies

Primary observational studies (case-control, cohort, and other population-based studies), in adults, on associations between the Pro12Ala polymorphism of PPAR $\gamma$ 2 gene (either separately or in combination with other markers) and the clinical outcome of interest for the respective objectives.

### Search methods

Electronic searches will be performed using, EMBASE, BIOSIS, Science Citation Index, LocusLink and PubMed. The search will not be limited to the English language, characteristics of investigators, or type of report. Reference lists of all relevant studies, and meta-analyses, will be examined to identify any additional studies. Investigators in the field will also be contacted for references to studies not yet identified.

Searches for articles pertaining to the association between Pro12Ala and T2DM will be conducted using the terms (1 OR 2 OR 3) AND (4 OR 5...OR 14) AND (15 OR 16 OR...21).

- 1- PPAR\*
- 2- Peroxisome proliferator activated receptor
- 3- Peroxisome\*
- 4- Proline
- 5- Pro
- 6- Alanine
- 7- Ala
- 8- Pro12ala
- 9- Pro/ala
- 10- x/ala
- 11- P12A
- 12- AA
- 13- PA
- 14- PP
- 15- Type 2 diabet\*
- 16- Hyperglycemia
- 17- Diabet\*
- 18- Type II diabet\*
- 19- NIDDM
- 20- Non-insulin dependent diabet\*
- 21- T2DM

## **Study selection**

All articles identified by the search will be scanned on the basis of title, keywords and abstract. Full texts will be assessed if faced with some uncertainty over inclusion by the title, keywords or abstract. All articles will be evaluated by two reviewers to assess eligibility or inclusion. If disagreement regarding an article exists a third reviewer will be consulted. Articles lacking information that cannot be obtained from the investigators will be excluded.

## **Quality assessment**

Methodological quality of each study to be included will be assessed according to the considerations described by Little *et al.* (27).

## **Data extraction**

Descriptive data will be extracted and summarised on participants, study design, outcome measures and genetic methodology (DNA extraction, genotyping assay, PCR conditions use of internal laboratory controls, laboratory technician blinding). A 3x2 table of results by genotype from each study will be sought from the literature, and if needed, from the investigators. A form will be designed to summarize all the above information from each study. Data will be extracted independently and in duplicate by two reviewers who shall use the same standardised data extraction form. Any disagreements will be adjudicated by a third reviewer.

## **Data synthesis**

Following the advice of Risch and Merikangas, Altshuler's *et al.* (19) TDT study assumed a multiplicative model of inheritance, 'this being the simplest model for which to calculate power' (23). The meta-analysis portion of their study did not, however, explicitly state a genetic model. A

recent meta-analysis investigating the association of Pro12Ala to obesity performed multiple pairwise comparisons, leading the authors to suggest that the mode of inheritance of Pro12Ala in obesity is recessive (24).

We will implement the approach of Minelli *et al.* (21), who have developed a model-free method for meta-analysis of genetic association studies. Using a parameter  $\lambda$ , one is able to capture information from the data about the mode of inheritance.  $\lambda$  is expected to remain constant across studies. The value of  $\lambda$  is unbounded but values below 0 and above 1 correspond to homozygous and over-dominant respectively. Values close to 0, 0.5 and 1 correspond to recessive, co-dominant and dominant respectively. It is expected that estimates of  $\lambda$  may fall anywhere between these numbers and this may represent the influence of other genetic and environmental effects.

If  $\lambda$  is not found to be constant across all studies a pairwise comparison using a bivariate random-effects meta-analysis that is capable of taking into account the within-study correlation will be applied. If  $\lambda$  is constant then the meta-analysis will be performed by standard methods as inverse-variance weighted averages using both fixed effects and random effects. Funnel plots will be used to investigate relationships between effect size and precision. Depending on this analysis, the appropriate meta-analysis (random or fixed) will be presented. Heterogeneity of results will be visualized in forest plots and quantified by the  $I^2$  statistic that describes the percentage of total variation due to heterogeneity as opposed to chance.

### *Potential conflict of interest*

None known

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