

The Peroxisome Proliferator-Activated Receptor- γ 2 Pro12Ala Polymorphism

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Peroxisome proliferator-activated receptor (PPAR)- γ is a transcription factor with a key role in adipocyte differentiation. The Ala allele of the common Pro12Ala polymorphism in the isoform PPAR- γ 2 is associated with reduced risk for type 2 diabetes. The effect on the individual is weak, but because of a prevalence of >75% of the high-risk Pro allele, the population-attributable risk is enormous. The in vivo effects of the polymorphism are secondary to alterations in adipose tissue, where PPAR- γ 2 is predominantly expressed. Moderate reduction in transcriptional activity of PPAR- γ as a result of the polymorphism modulates production and release of adipose-derived factors. Both decreased release of insulin-desensitizing free fatty acids, tumor necrosis factor- α , and resistin and increased release of the insulin-sensitizing hormone adiponectin result in secondary improvement of insulin sensitivity of glucose uptake and suppression of glucose production. The population effect of this polymorphism may be modulated by environmental or genetic factors such as obesity, ethnicity, ratio of unsaturated to saturated fatty acids, and genetic background. Once diabetes has developed, the protective effect of the Ala allele may be lost, since increased vascular complications and more pronounced β -cell dysfunction have been reported. These observations, however, are currently unexplained. In conclusion, the Pro12Ala polymorphism in PPAR- γ 2 represents the first genetic variant with a broad impact on the risk of common type 2 diabetes. The precise understanding of its mechanism may lead to novel diagnostic, preventive, and therapeutic approaches for improving the management of type 2 diabetes. *Diabetes* 51: 2341–2347, 2002

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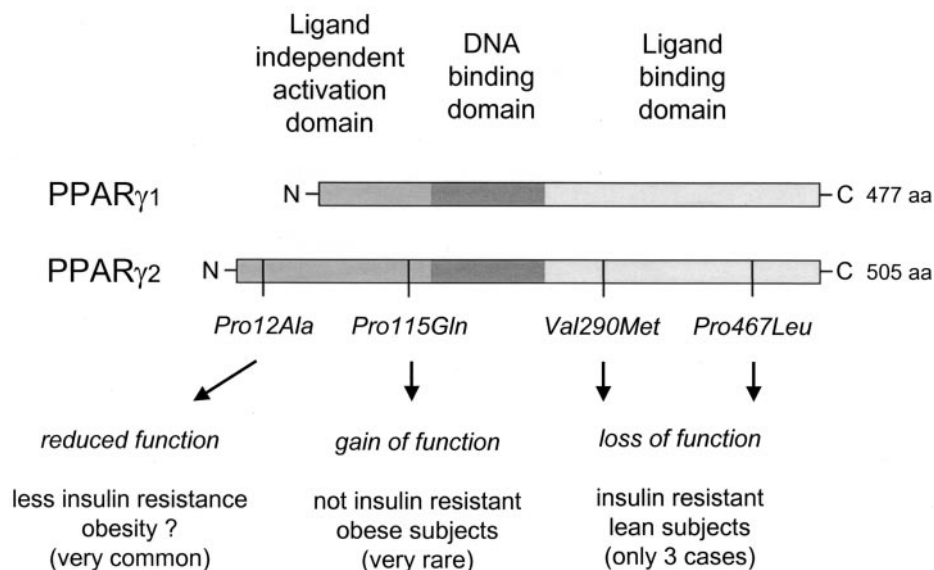
FFA, free fatty acid; PPAR, peroxisome proliferator-activated receptor; TNF- α , tumor necrosis factor- α .

Peroxisome proliferator-activated receptor (PPAR)- γ is a transcription factor that belongs to the same family of nuclear receptors as steroid and thyroid hormone receptors (1). It is activated by certain fatty acids, prostanoids, and thiazolidinediones, a novel class of insulin-sensitizing antidiabetic agents (2–4). Upon activation, it heterodimerizes with the retinoid X receptor and binds to specific PPAR-responsive elements of DNA to promote transcription of numerous target genes (5). Although the isoform PPAR- γ 1 is expressed in most tissues, PPAR- γ 2 is specific for adipose tissue, where it plays a key role in regulating adipogenic differentiation (6). The PPAR- γ gene is located on chromosome 3 (7), and the specific isoforms are a result of alternative mRNA splicing.

A number of genetic variants in the PPAR- γ gene have been identified. These include a very rare gain-of-function mutation (Pro115Gln) associated with obesity but not insulin resistance (8), two loss-of-function mutations (Val290Met and Pro467Leu) reported in three individuals with severe insulin resistance but normal body weight (9), the silent CAC478CAT mutation (10–12), and the highly prevalent Pro12Ala polymorphism in PPAR- γ 2 (Fig. 1). The latter is the result of a CCA-to-GCA missense mutation in codon 12 of exon B of the PPAR- γ gene. This exon encodes the NH₂-terminal residue that defines the adipocyte-specific PPAR- γ 2 isoform. The Pro12Ala polymorphism in PPAR- γ 2, which is the focus of this review, was first identified in 1997 (13), and the rare allele frequencies are ~12% in Caucasians, 10% in Native Americans, 8% in Samoans, 4% in Japanese, 3% in African-Americans, 2% in Nauruans, and 1% in Chinese (14,15). In Caucasians, the ethnic group with the highest frequency, this translates into a carrier prevalence of the polymorphism of almost 25%.

ASSOCIATION WITH TYPE 2 DIABETES

The pathogenesis of type 2 diabetes is characterized by failure of β -cell function to compensate for decreased insulin sensitivity. The etiology is multifactorial, and twin studies clearly indicate a major role for involvement of genetic factors (16,17). Moreover, excess concordance rates in monozygotic versus dizygotic twins clearly suggest a contribution of genetic factors to both insulin resistance and β -cell dysfunction (18). However, due to enormous heterogeneity and probably polygenicity, very

FIG. 1. Organization of the PPAR- γ gene.

few genetic variants have been identified to account for a substantial proportion of common type 2 diabetes.

First evidence for an association between the Pro12Ala polymorphisms in PPAR- γ 2 and type 2 diabetes came from Japanese-Americans, in which a frequency of the rare Ala allele of 9.3% in subjects with normal glucose tolerance versus only 2.2% in patients with type 2 diabetes was observed (11). This type of association study, however, is frequently troubled by a lack of reproducibility. Nevertheless, in a study that used the powerful tool of transmission disequilibrium testing in 333 Scandinavian parent-offspring trios with abnormal glucose tolerance, only the Pro12Ala polymorphism remained significant among 16 variants with previously published evidence for association with type 2 diabetes (or related disorders) (19). This association of the Pro12Ala polymorphism with type 2 diabetes was recently replicated in two large population-based studies from Finland and Japan including well over 4,000 subjects (15,20).

Although the initial publication reported a 75% risk reduction for diabetes conferred by the Ala allele (11), of five subsequent studies (12,21–24), only one (22) was able to reproduce a significant association. However, a meta-analysis including those five plus the aforementioned data set (19) demonstrated a significant risk reduction of 21% (19). The resulting population-attributable risk was estimated as \sim 25% (11). In other words, if the entire population carried the Ala allele, the prevalence for type 2 diabetes would be 25% lower. In contrast, mutations responsible for monogenic mature-onset diabetes of the young, though having a major effect on the individual's glucose homeostasis, are so rare that they hardly affect a population's risk of type 2 diabetes (25). This underlines the importance of alleles with weak individual effect but high population prevalence, such as the (so-called) wild-type Pro allele of PPAR- γ 2 (75% prevalence in Caucasians), and clearly indicates the prominent role of PPAR- γ among candidate genes for common type 2 diabetes.

To make diagnostic, preventive, or therapeutic use of a polymorphism, it is necessary to understand how and in which metabolic, genetic, or environmental context the genotype influences the phenotype. A number of recent

studies on metabolic pathways, diabetic and nondiabetic subphenotypes, and gene-environment and gene-gene interaction effects have provided some useful information regarding the potential mechanism by which the Pro12Ala polymorphism reduces the risk for type 2 diabetes. Generally, a genetic variant that affects the risk for type 2 diabetes must influence insulin sensitivity, insulin secretion, or susceptibility for obesity.

EFFECT ON INSULIN SENSITIVITY

In the original article, significantly greater insulin sensitivity was reported in nondiabetic Ala carriers (11). However, Ala carriers also had a lower BMI, and after adjustment the difference in insulin sensitivity was no longer significant. It therefore remained unclear what the primary result of the polymorphism was. In one small study, the confounding influence of BMI (and other relevant variables) on insulin sensitivity was eliminated by pair-matching carriers of the polymorphism with wild-type control subjects (26). Glucose infusion rates during euglycemic-hyperinsulinemic clamp (the gold standard technique for measuring insulin sensitivity in humans) per se were not different; however, expressing them per unit insulin revealed a significantly greater insulin sensitivity index (26). In the largest study so far addressing this issue, a 7% greater insulin sensitivity was observed in 616 normal glucose-tolerant Swedish 70-year-old men carrying the Ala allele (27). In the same article, a 6% difference in the same direction using the minimal model estimate for insulin sensitivity was reported for 364 young Danes; however, it failed to reach statistical difference. A power calculation based on these data revealed a required sample size of \sim 2,000 to achieve significance. In a Chinese/Japanese sib-pair study, which by design controlled relatively well for genetic background and childhood environment, a greater estimated insulin sensitivity (homeostasis model analysis) was observed in carriers of the Ala allele (28). Interestingly, a more pronounced effect of the Pro12Ala polymorphism on insulin sensitivity was observed before the background of the common Gly972Arg polymorphism in insulin receptor substrate-1 (29). Taken together, these studies provide

substantial evidence that the Pro12Ala polymorphism improves insulin sensitivity in humans.

In subgroups with obesity, the difference in insulin sensitivity was more pronounced (22,30,31), suggesting an interaction of the polymorphism with factors originating from adipose tissue. In healthy, lean, and normal glucose tolerant subjects, greater insulin sensitivity of glucose disposal was tightly coupled to greater insulin sensitivity of lipolysis, as measured by isotope dilution (32). This finding was in principle confirmed in a larger cohort from the same laboratory by demonstrating significantly lower free fatty acid (FFA) concentrations during insulin stimulation (33). Based on these results, it appears possible that alterations in transcriptional activity of the Ala variant in adipocytes (where PPAR- γ 2 is expressed) primarily enhance insulin's action on suppression of lipolysis, resulting in a decreased release of FFAs. Secondly, reduced availability of FFAs would then permit muscle to utilize more glucose and liver to suppress glucose production more efficiently upon insulin stimulation (34). The metabolic studies in humans were not designed to distinguish between insulin-sensitizing effects on peripheral glucose disposal and suppression of glucose production. Studies are therefore required that specifically address the question of whether this polymorphism affects glucose production and insulin clearance, two processes clearly influenced by FFA availability.

EFFECT ON INSULIN SECRETION

Currently, strong evidence for a direct effect of the Pro12Ala polymorphism on insulin secretion is lacking. However, in Japanese subjects with manifest type 2 diabetes, a lower β -cell function index (homeostasis model analysis) was reported in carriers of the Ala allele (15). Interestingly, lipid infusion designed to elevate plasma FFA concentrations fourfold resulted in a decrease in insulin secretion during hyperglycemic clamp in carriers of the Ala allele, but an increase in control subjects with two Pro alleles (35). These findings might provide a partial explanation for the above observation that β -cell function deteriorates more in Ala carriers once diabetes has developed (15). Conceivably, superimposition of secondary mechanisms—such as chronic exposure to elevated FFAs and/or hyperglycemia, which are characteristic for overt type 2 diabetes—alters (or even reverses) the effect of the genetic variant. Expression of PPAR- γ , though not specifically of the isoform PPAR- γ , in β -cells has been demonstrated (36). However, whether any of the above effects on β -cell function were direct or secondary remains open and requires further and more detailed studies in, for example, heterozygous PPAR- γ knockout animals or humans homozygous for the Ala allele.

EFFECT ON OBESITY

Because PPAR- γ plays a key role in adipocyte differentiation and body fat mass is a strong determinant of insulin sensitivity and type 2 diabetes, the influence of the Pro12Ala polymorphism on susceptibility for obesity has been of major interest. In Pima Indians, suggestive linkage (logarithm of odds = 2.0) with percentage body fat was reported for the 3p24.2-p22 locus, which is close to the region harboring the PPAR- γ gene (3p25-p24.2) (37).

Cross-sectional studies with small to moderate sample sizes yielded inconsistent results by demonstrating either no difference (21,23,28,38,39) or a modestly greater BMI in carriers of the Ala allele (10,40,41). Two sufficiently large studies (>1,000 nondiabetic subjects) found a lower BMI in Finns (11) and no difference in Japanese (15). On the other hand, longitudinal studies in selected populations with relatively small sample sizes consistently suggested greater weight gain in association with the Ala allele (20,42,43).

Thus, although cross-sectional evidence convincingly argues against susceptibility of obesity conferred by the Ala allele, the issue remains somewhat unclear. In particular, the interpretation of the longitudinal analysis is complicated by the fact that greater insulin sensitivity per se predicts future weight gain (44). Weight gain may be related to the greater insulin sensitivity particular of lipolysis (33), which would result in overproportionate retention of FFAs in stored triglycerides during physiological insulin stimulation (e.g., postprandially). In this scenario, obesity would be a consequence of increased insulin sensitivity and not directly of the Ala allele. It may be of note in this context that under a high-fat diet, heterozygous PPAR- γ -deficient mice (an animal model for reduced PPAR- γ activity) were protected from the development of insulin resistance caused by adipocyte hypertrophy, compared with wild-type littermates (45).

Clearly, any effect of this polymorphism on measures of obesity would be extremely subtle, and its demonstration would be crucially dependent on subject selection and interaction with ethnic background and other genetic or environmental factors. For example, an interaction effect of the Pro12Ala polymorphism with the Trp64Arg polymorphism in the β_3 -adrenergic receptor (which by itself probably does not influence body weight [46]) has been reported (47). Moreover, a greater BMI was observed in Ala carriers when the dietary polyunsaturated fat-to-saturated fat ratio was low, suggesting gene-nutrient interaction via the PPAR- γ locus (48).

CELLULAR MECHANISM

PPAR- γ is a master transcriptional regulator involved in the expression of probably hundreds of genes (1). Two studies have directly examined the transcriptional activity of the Ala variant of PPAR- γ in comparison to the Pro variant in experimental cell models (11,49). Reduced binding of the Ala variant to the PPAR- γ -responsive DNA elements was observed in transient transfection assays (11,49). Moreover, reduced transcription of specific genes (lipoprotein lipase and acyl-CoA oxidase) was reported for cells overexpressing the Ala variant compared with cells overexpressing the wild-type protein (11). These studies clearly indicate reduced transcriptional activity of PPAR- γ as a result of the Pro-to-Ala exchange. Because PPAR- γ 2 is exclusively expressed in fat cells, any metabolic effects of the polymorphism, including those on glucose homeostasis, are likely to be secondary to alterations in adipose tissue.

There is evidence from humans that the Pro12Ala polymorphism promotes the suppression of FFA release by insulin (32,33). It is unclear, however, which genes are specifically affected by the transcriptional changes con-

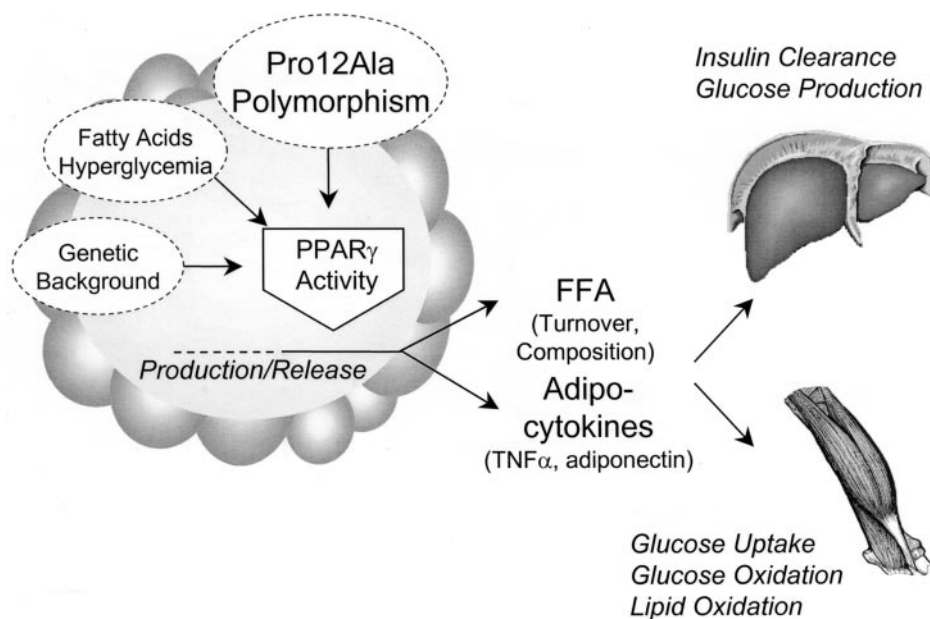


FIG. 2. Effect of the Pro12Ala polymorphism and other factors on PPAR- γ activity in adipocytes and release of mediators relevant for glucose homeostasis.

ferred by the polymorphism. Conceivably, the proportion of large versus small adipocytes is involved. Activators of PPAR- γ have been shown to promote differentiation of preadipocytes to small adipocytes (50), and in small adipocytes lipolysis is more insulin sensitive than in large adipocytes (51). In addition, alterations in fat distribution (more subcutaneous and less visceral [52]) as a result of the polymorphism could mediate effects on lipolysis. In humans, PPAR- γ expression in visceral adipose tissue relative to subcutaneous adipose tissue is increased in obese subjects (53). Because visceral adipose tissue is metabolically more harmful (54), the Ala allele would be expected to have an even greater impact in obese subjects, as in fact was shown to be the case (30). Interestingly, heterozygous PPAR- γ -deficient transgenic mice had smaller adipocytes and greater insulin sensitivity than wild-type mice (45). These mice were also characterized by greater insulin sensitivity of both glucose disposal and suppression of glucose production (45,55). In these studies, insulin suppression of FFA release was not determined but would represent a prime candidate for mediating the insulin effects on glucose homeostasis.

In addition to fatty acids, adipose tissues release a number of peptide hormones that influence insulin sensitivity. These include the cytokine tumor necrosis factor- α (TNF- α) (56), resistin (57), and adiponectin (apM-1) (58). Although the insulin desensitizing evidence for TNF- α and resistin is weak for humans, adiponectin concentrations were clearly shown to be positively correlated with insulin sensitivity, even after adjusting for body fat (59), to decrease with deteriorating glucose tolerance (59), and to increase after weight reduction (60). Moreover, intravenous administration of recombinant adiponectin to rodent models of insulin resistance restored normal insulin sensitivity (61). Because all three peptides are under transcriptional control of PPAR- γ (57,62–64), any of them (and possibly other peptides yet to be identified) could mediate the Pro12Ala effect on insulin sensitivity (Fig. 2). In humans, however, it has not been studied yet whether the

Pro12Ala polymorphism influences systemic or local adipocytokine concentrations.

It has been difficult to explain why, paradoxically, both reduced transcriptional activity (due to heterozygous knockout or the Pro12Ala polymorphism) and pharmacological activation (by thiazolidinediones) of PPAR- γ results in improved insulin sensitivity (65). Recent studies in heterozygous PPAR- γ -deficient mice, however, have contributed to unraveling this problem. Alterations in white adipose tissue triglycerides, hepatic triglycerides, hepatic energy consumption, and muscle energy metabolism were markedly different between the two levels of PPAR- γ activation (63), indicating that entirely different metabolic pathways must be mediating the insulin-sensitizing effect of supraphysiological stimulation (thiazolidinediones) and moderate reduction (heterozygous knockout or Pro12Ala) of PPAR- γ activity.

Comparison with other naturally occurring variants in PPAR- γ may be useful for elucidating the molecular mechanisms. The Val290Met and Pro467Leu mutations drastically reduced transcriptional activity of PPAR- γ in a dominant-negative fashion in vitro and resulted in a severely insulin resistant though lean phenotype in vivo (9). Structure-function simulations suggested that these mutations affect the orientation of helix 12 of PPAR- γ , which is important for the interaction with ligands and coactivators (9). On the other hand, these mutations are less likely to affect ligand-independent transcriptional activity, a process strongly controlled by the NH₂ terminus (66), the domain that harbors codon 12. Thus, the net result of an amino acid exchange that decreases transcriptional activity of PPAR- γ critically depends on the localization within the PPAR- γ molecule. In support of this concept, antidiabetic properties of PPAR- γ antagonists (67) or PPAR- γ modulators (68–70) have been demonstrated.

Finally, it is necessary to point out that the CCA-to-GCA missense mutation in PPAR- γ (which causes the Pro12Ala exchange) need not necessarily be the functionally relevant mutation, but rather in linkage disequilibrium with it.

For example, the functional mutation could reside in the promoter region of the PPAR- γ gene and result in reduced expression of PPAR- γ protein. The Pro12Ala polymorphism would then merely represent a genetic marker for the relevant mutation. However, the demonstrated reduction in transcriptional activity argues against such a scenario (11,49). In addition, a systematic screening of 70 diabetic individuals failed to reveal additional missense mutations elsewhere in the PPAR- γ gene or its promoter (19). All in all, a strong case exists for Pro12Ala itself being the etiologic exchange.

SUMMARY AND CONCLUSIONS

The transcription factor PPAR- γ is a master regulator of the relationships between nutrients, susceptibility to obesity, control of peptides released from adipocytes, and insulin sensitivity. The alanine allele of the common Pro12Ala polymorphisms in the isoform PPAR- γ 2 is associated with a 25% reduced risk for type 2 diabetes. It thus represents the first genetic variant with a broad impact on the risk of common type 2 diabetes. The effect of this polymorphism is probably mediated by increased insulin sensitivity, which may be secondary to more efficient suppression of FFA release from fat tissue, where the isoform PPAR- γ 2 is exclusively expressed. Modulation of expression and release of adipocytokines that influence insulin sensitivity are likely also to be involved, but this remains to be demonstrated in humans. The effects on muscle, liver, and possibly other tissues ultimately influencing glucose homeostasis are secondary. The underlying molecular mechanism of this polymorphism is a moderate reduction of the ligand-independent activity of PPAR- γ . Some findings for the Pro12Ala variant differed depending on superimposition of environmental factors. This clearly suggests that so-called "gene-environment interaction" may well hinge on genetic variations, such as those occurring in the transcription factor PPAR- γ . A number of issues remain to be confirmed and further explored, including the increased vascular complications and more pronounced β -cell dysfunction associated with the Ala allele once diabetes has developed. Moreover, the likely interaction with independent modulators such as obesity, ethnicity, ratio of unsaturated to saturated fatty acids, and other common genetic polymorphisms requires further studies. The understanding of how specific modulation of PPAR- γ influences metabolism in humans may accelerate the development of novel pharmacological agents useful for preventing or treating type 2 diabetes and related disorders.

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