Original Article

A Possible Role for the *PPARG* Pro12Ala Polymorphism in Preterm Birth

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The links between preterm birth, low birth weight, and adult vascular/metabolic morbidity remain unclear. Genetic susceptibility of babies related to these three conditions might contribute to this long-term association. We tested whether the Pro12Ala polymorphism of the peroxisome proliferator–activated receptor γ (*PPARG*) gene could play a role in birth weight and duration of gestation. We genotyped two independent cross-sectional studies from Northern Ireland (n = 382 and 620). In combined populations, the *PPARG* Ala12 allele was associated (P = 0.03) with lower birth weight, primarily caused by shorter gestational duration (P = 0.04). The frequency of Ala12 allele carriers was higher (P = 0.027) in the group of individuals born before term (35%, n = 60) than in the group of individuals born at term (22%, n = 942). The odds ratios (95% CI) of preterm birth for Ala12 allele carriers were 1.9 (1.1–3.4), P = 0.022, and 4.2 (1.9–9.7), P = 0.0006 (adjusted for sex, maternal age, and study), when considering 37 or 35 weeks of pregnancy as a threshold for preterm birth, respectively. Interestingly, the same allele was also associated with a moderate decreased risk of miscarriages in mothers. In conclusion, the *PPARG* Pro12Ala polymorphism might represent a genetic susceptibility factor for preterm birth and constitute a link between preterm birth and metabolic diseases later in life.
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Several studies suggest that a small size at birth (due to poor fetal growth and/or preterm delivery) is associated with substantially elevated risks of metabolic syndrome (dyslipidemia, insulin resistance, and hypertension), type 2 diabetes, and cardiovascular disease in adulthood (1–3). A detrimental intrauterine development or fetal environment (inadequate supply of nutrients or oxygen) has been proposed to explain this association. By adapting themselves to a limited supply of nutrients, fetuses improve their short-term survival but permanently change their physiology and metabolism. In addition to these environmental factors, a common genetic predisposition, variably expressed at different stages of life, may explain the association between preterm delivery and adult vascular and metabolic diseases. Thrifty genotypes, helping to counter the detrimental intrauterine environment, may be preferentially selected in survivors (4). For instance, a pleiotropic transcriptional factor, subject to environmental influences throughout life may constitute such a possible link. In this context, the peroxisome proliferator–activated receptor γ (PPARG), a member of the nuclear hormone receptor subfamily that plays a major role in adipose tissue differentiation and in susceptibility to type 2 diabetes in humans, may represent a potential candidate gene. PPARG forms heterodimers with retinoid X receptor, which regulate transcription of various genes. A common polymorphism in the *PPARG* gene (Pro12Ala, rs1801282) at codon 12 of exon B has been associated with a modification of the risk of developing type 2 diabetes as well as fat mass control in various adult populations (5). We tested whether the *PPARG* Pro12Ala polymorphism was associated with birth weight and gestational duration into a sample constituted by a cohort of 21- to 25-year-old men and women (YH3, n = 382) and a cohort of 15-year-old boys and girls (YH2000, n = 620) from Northern Ireland.

RESEARCH DESIGN AND METHODS

Populations. The Young Hearts (YH) project is a prospective study investigating the development of biological and behavioral risk factors for cardiovascular disease in an adolescent population in Northern Ireland. Details of the study design and sampling procedure have been presented elsewhere (6). Briefly, in 1989–1990, a 2% representative sample of school children aged 12 and 15 years in Northern Ireland (YH3, n = 1,015) was collected. The original 12-year-old population was followed up in 1992–1993 (YH2) with complete data collected on 225 boys and 230 girls (90% response rate). Between 1997 and 1999, all original YH participants were invited to participate in the third screening phase (YH3, age 21–25 years, n = 489), and a blood sample for DNA extraction was taken at that time.

A further cross-sectional survey, the Young Hearts 2000 (YH2000), was carried out in 2000. Approximately 2,000 boys and girls aged 12 and 15 years (500 in each of the four age-sex groups) were recruited through postprimary schools. Details of the study design have been presented elsewhere (7).

In Northern Ireland, birth records have been computerized since 1971 by the Department of Health and Social Services. Records concerning birth weight and length of gestation were available from this data system. Other perinatal information available included parity, parental age, number of miscarriages, and number of stillbirths. Genomic DNA and data on gestational duration and birth weight were available for 382 and 620 singleton births in YH3 and YH2000, respectively. The prevalence of preterm birth (before 37

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AGA, appropriate for gestational age; PPARG, peroxisome proliferator–activated receptor γ; SGA, small for gestational age; TNF-α, tumor necrosis factor-α.

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TABLE 1
Gestational duration and birth weight according to *PPARG* genotypes in the combined sample

	Crude			Adjusted			Adjusted		
	Pro12Pro	Ala12 carriers	*P*	Pro12Pro	Ala12 carriers	*P*	Pro12Pro	Ala12 carriers	*P*
n	767	229		767	229		767	229	
Birth weight (g)	3,427 ± 19	3,343 ± 35	0.04	3,377 ± 27	3,298 ± 39	0.05*	3,398 ± 23	3,363 ± 35	0.31†
Gestational age (weeks)	39.3 ± 0.1	39.0 ± 0.1	0.03	39.1 ± 0.1	38.8 ± 0.1	0.03*	39.2 ± 0.1	39.0 ± 0.1	0.13‡

Data are means or adjusted means ± SE. From the 1,002 subjects, data on maternal age were missing for 6 individuals. **P* values adjusted for sex, maternal age, and study. †*P* values adjusted for sex, maternal age, gestational duration, and study. ‡*P* values adjusted for sex, maternal age, birth weight, and study.

completed weeks of gestation) was 5.3 and 7.8% in the YH3 and YH2000 studies, respectively.

Ethical approval was obtained from the Research Ethics Committee of the Queen's University of Belfast, and written informed consent for participation was obtained from all participants and from each participant's parent or guardian.

Genetic analyses. Fasting blood samples (40 ml) were drawn from the antecubital vein. Genomic DNA was extracted from white blood cells, and DNA amplification of the *PPARG* Pro12Ala polymorphism was performed using the following oligonucleotides : forward, 5′-TCT GGG AGA TTC TCC TAT GG-3′, and reverse, 5′-CCC AAT AGC CGT ATC TGG AAG G-3′, generating a 99-bp amplicon (annealing temperature 52°C). To do the genotyping, a restriction enzyme site was forced into the forward primer to create a *Sau96*I site. Eight-microliter PCR products were digested with 1.5 units of *Sau96*I (New England Biolabs, Ozyme, France) at 37°C overnight. The *Sau96*I site was present in the case of the Pro12 allele, whereas the Ala12 allele destroyed it. The *Sau96*I enzyme digested the 99-bp PCR product into two fragments in the case of the Pro12 allele (80 + 19 bp) visualized on a 3% agarose gel. We double-checked the digestion efficiency for the uncut PCR products (corresponding here to the Ala12Ala homozygous individuals), and all Ala12Ala genotypes were confirmed ($n = 20$).

Statistical analyses. Statistical analyses were performed with the SAS statistical software, version 8 (SAS Institute, Cary, NC). Only singleton births were analyzed. Genotypic and allelic distributions according to sex and birth status were compared with Pearson χ^2, Fisher's exact, or Mantel-Haenszel tests when appropriate. Because of the low number of Ala12Ala homozygote subjects, the Pro12Ala polymorphism was considered as a dichotomous variable in the analyses: subjects carrying at least one Ala12 allele versus *Pro12Pro* subjects (dominant effect). The effect of the polymorphism on the risk of preterm birth was tested in a multiple logistic regression model and estimated by the odds ratio (OR). We categorized maternal age into three classes because it has been reported in the literature that the effect of maternal age on preterm delivery is not linear. A young or an old maternal age is a risk factor for preterm delivery. As a consequence, we took the 10% youngest and 10% oldest maternal age of the whole population to have contrasted classes of age rather than a continuous variable. The maternal age was therefore coded in three classes : ≤21, 21–36, and ≥36 years. We identified, among individuals born at term, the individuals born small for gestational age (SGA, $n = 23$), i.e., individuals with a birth weight below the 3rd percentile for sex and gestational age, and the individuals who were born appropriate for gestational age (AGA, $n = 449$), i.e., individuals born with birth size between the 25th and 75th percentiles (8). Interactions between genotypes and covariates were tested. The homogeneity of the ORs between cohorts was assessed with the Breslow-Day test. Statistical significance was considered at $P < 0.05$.

RESULTS

The genotype distribution of the Pro12Ala polymorphism in YH3 ($n = 382$) was Pro12Pro 76.9%, Pro12Ala 22.1%, and Ala12Ala 1.0% and was not different from the Hardy-Weinberg expectations ($\chi^2 = 0.14$, $P = 0.71$). In YH2000 ($n = 620$), the genotype distribution of the Pro12Ala polymorphism was Pro12Pro 77.0%, Pro12Ala 21.2%, and Ala12Ala 1.8% and was not different from the Hardy-Weinberg expectations ($\chi^2 = 0.29$, $P = 0.59$). The frequency of the Ala12 allele was 12% in both cohorts.

We looked at the impact of the Pro12Ala polymorphism on birth weight and gestational duration in the combined sample ($P = 0.14$ and 0.08, respectively, for the test of heterogeneity within cohorts). The birth weight of the carriers of the Ala12 allele was significantly lower than birth weight of the carriers of the Pro12Pro genotype in the combined sample (3,343 ± 35 vs. 3,427 ± 19 g in Ala12 allele carriers and Pro12Pro, respectively, $P = 0.04$) (Table 1). This result was little altered by adjustment for sex, maternal age, and study; an additional adjustment for gestational duration attenuated the association. The correlation coefficient between gestational duration and birth weight was high, at $r^2 = 0.21$ ($P < 0.0001$). Gestational duration was shorter in Ala12 allele carriers than in Pro12Pro individuals ($P = 0.03$). Similarly, this result was attenuated ($P = 0.13$) by adjustment for birth weight, sex, maternal age, and study (Table 1). Therefore, the association between the Pro12Ala polymorphism and birth weight seemed to be the consequence of an association between the Pro12Ala polymorphism and gestational duration.

We then stratified the individuals according to their births being at term (after 37 weeks of gestation, $n = 942$) or before term (before 37 completed weeks of gestation, $n = 60$). The mean birth weight and gestational duration in preterm or at-term individuals were similar in both cohorts (Table 2). We compared the distribution of the Pro12Ala polymorphism in children born before and at term (Table 3). There was no strong evidence of heterogeneity between studies ($P = 0.17$), allowing the pooling of the two cohorts. There were 35% of Ala12 allele carriers in the group of individuals born before term (before 37 weeks of gestation) compared with 22% of Ala12 allele carriers in the group of individuals born at term (after 37 weeks of gestation) ($P = 0.027$). The OR (95% CI) of preterm birth was 1.9 (1.1–3.4), $P = 0.022$, for Ala12 allele carriers after

TABLE 2
Gestational duration and birth weight according to preterm birth status in YH3, YH2000, and combined sample

	YH3		YH2000		Combined	
	At term	Before term	At term	Before term	At term	Before term
n	363	19	579	41	942	60
Gestational age (weeks)	39.7 ± 1.0	34.1 ± 1.9	39.4 ± 1.1	34.7 ± 1.7	39.6 ± 1.1	34.5 ± 1.8
Birth weight (g)	3,464 ± 491	2,501 ± 455	3,463 ± 466	2,513 ± 589	3,463 ± 476	2,503 ± 550

Data are means ± SD.

TABLE 3
PPARG Pro12Ala polymorphism frequency according to birth status in the combined sample

PPARG Pro12Ala	Pro12Pro	Pro12Ala	Ala12Ala	*P**	Pro12Pro	Ala12 carriers	*P*	OR (95% CI)
At term (n = 942)	731 (77.6)	196 (20.8)	15 (1.6)		731 (77.6)	211 (22.4)		1.9 (1.1–3.4)
Before term (n = 60)	39 (65.0)	20 (33.3)	1 (1.7)	0.06	39 (65.0)	21 (35.0)	0.027	P = 0.022

Data are n (%). *Fisher's exact test. The OR was adjusted for sex, maternal age, and study.

adjustment for sex, maternal age, and study. When the cohorts were considered independently, the impact of the *PPARG* Pro12Ala polymorphism on preterm birth had adjusted ORs for Ala12 carriers of 3.5 (1.3–9.4), P = 0.012, and 1.4 (0.7–2.9), P = 0.33, in YH3 (19 preterm vs. 363 at-term individuals) and YH2000 (41 preterm vs. 579 at-term individuals), respectively.

We then examined the *PPARG* Pro12Ala polymorphism frequency according to weeks of gestation (from 44 to 30 weeks) (Table 4). The Ala12 allele frequency increased dramatically in the groups composed of individuals born before 35 weeks of gestation compared with the groups of individuals born after 35 weeks of gestation. When taking a threshold for preterm birth below 35 weeks of gestation, the sex- and maternal age–adjusted ORs (95% CI) of preterm birth for Ala12 carriers were 5.0 (1.2–19.9) (P = 0.02), 4.0 (1.4–11.3) (P = 0.009) and 4.2 (1.9–9.7) (P = 0.0006) in YH3, YH2000, and the combined sample, respectively (Table 4; data not shown).

We also compared the *PPARG* Pro12Ala polymorphism genotype distribution between individuals born at term but either SGA (n = 23) or AGA (n = 449) (see RESEARCH DESIGN AND METHODS). There were 349 (77.7%) Pro12Pro and 100 (22.3%) Ala12 allele carriers in the AGA group compared with 13 (56.5%) Pro12Pro and 10 (43.5%) Ala12 allele carriers in the SGA group (P = 0.023) (data not shown). The OR of being born SGA for Ala12 allele carriers was 2.7 (1.1–6.3), P = 0.023, and did not differ after adjustment for sex, maternal age, and study (2.5 [1.1–5.9], P = 0.05). Importantly, when removing the 23 SGA individuals from the group of individuals born at term, the adjusted OR of preterm birth for Ala12 allele carriers remained 2.1 (1.2–3.6), P = 0.01, suggesting that the present association with preterm birth was independent of being born SGA.

Finally, we examined the *PPARG* Pro12Ala polymorphism frequency according to the mother-reported number of miscarriages and previous stillbirths (Table 5). The Ala12 allele was significantly less frequent in the miscarriage groups than in the group without miscarriage (Mantel-Haenszel test, P = 0.03). There was a consistent trend toward a lower Ala12 allele frequency in the stillbirth (9.1%) compared with the live birth (22.9%) groups, but it did not reach significance (P = 0.19).

DISCUSSION

Our data showed that the Ala12 allele of the *PPARG* Pro12Ala polymorphism was associated with lower birth weight due to shorter gestational duration. In addition, the risk of being born before 37 and 35 weeks of gestation was two- and fourfold higher, respectively, for Ala12 allele carriers compared with Pro12Pro individuals. These results were consistent for two independent studies from Northern Ireland.

The prevalence of preterm delivery (before 37 completed weeks of gestation) was 5.3 and 7.8% in theYH3 and YH2000 studies, respectively. It has been shown that preterm delivery prevalence varies from 5 to 15% of all deliveries, depending on the population studied (rev. in 9). Because preterm delivery of infants remains one of the most intractable problems contributing to perinatal morbidity and mortality in obstetric practice in developed countries, it represents a public health concern. Preterm births account for more than two-thirds of all singleton neonatal deaths excluding congenital malformations (10). The general knowledge of the causes of preterm delivery is limited. It seems that 30–50% of the cases are due to a spontaneous preterm labor, whereas the others are essentially due to multiple gestation, preterm labor rupture of membranes, or hypertension. Preterm delivery is influenced by genetic and environmental determinants (11). Twin studies have estimated the heritability of preterm delivery to be 27%, implying that genetic factors may affect the gestational duration (12).

We have shown that individuals carrying the *PPARG* Ala12 allele have a higher risk of being born prematurely than *Pro12Pro* individuals. *PPARG* is not the first genetic factor suspected in preterm birth in humans. It has been previously shown that increased amniotic fluid concentrations of tumor necrosis factor-α (TNF-α) are observed in women with preterm labor and subsequent preterm birth (13,14). The impact of the −308 G/A polymorphism located in the TNF-α gene promoter, known to induce the transcription of the TNF-α gene, has been studied in relation to preterm delivery. One study failed to find an association between the −308 G/A polymorphism and preterm delivery (15). In contrast, four other studies

TABLE 4
PPARG Pro12Ala polymorphism frequency according to weeks of gestation in the combined sample

Gestational age (weeks)	44–42	41	40	39	38	37	36–35	34–33	32–30
n (total = 1,002)	20	128	420	203	127	44	36	15	9
Pro12Pro	17 (85.0)	103 (80.5)	316 (75.2)	159 (78.3)	99 (78.0)	77 (84.1)	28 (77.8)	7 (46.7)	4 (44.4)
Ala12 carriers	3 (15.0)	25 (19.5)	104 (24.8)	44 (21.7)	28 (22.0)	7 (15.9)	8 (22.2)	8 (53.3)	5 (55.6)
OR (95% CI)*	1.0 (reference)							4.0 (1.4–11.2)	4.8 (1.2–18.5)
Global OR (95% CI)*	1.0 (reference)							4.2 (1.9–9.7)	

Data are n (%). *Adjusted for sex, maternal age and study.

TABLE 5
PPARG Pro12Ala polymorphism frequency according to numbers of previous stillbirths and miscarriages in mothers

	0 Miscarriage	1 Miscarriage	>1 Miscarriage	*P**	0 Stillbirth	1 Stillbirth	*P*†
Pro12Pro	495 (76.1)	126 (79.3)	41 (91.1)		639 (77.1)	20 (90.9)	
Ala12 allele carriers	155 (23.9)	33 (20.7)	4 (8.9)	0.03	190 (22.9)	2 (9.1)	0.19
Total	650	159	45		829	22	

Data are *n* (%). *Mantel-Haenszel χ^2 test. †Fisher's exact test.

showed that the −308 G allele was associated with an increased risk of preterm birth (16–19). Associations were also found between the *β2-adrenergic receptor* (ADRB2) polymorphisms and preterm labor (20,21). Moreover, Landau et al. (22) showed that *ADRB2* Arg16 homozygosity improves pregnancy outcome after β(2)-agonist tocolysis. All of these studies confirm the importance of genetic factors in preterm delivery.

In our study, we also found an association between the *PPARG* Ala12 allele and a 2.5-fold increase in the risk of being born SGA among individuals born at term, although caution must be taken concerning these data because only 23 SGA individuals were considered. The association between the Ala12 allele and preterm birth or SGA were independent from each other, reinforcing the hypothesis that the Ala12 allele could be associated with a deleterious intrauterine environment, promoting either preterm birth or being born SGA among individuals born at term.

In vitro experiments demonstrate that the PPARG receptor with alanine at position 12 is less transcriptionally active than the receptor with proline (23,24). A recent study showed that the Ala12 allele was associated with greater insulin-mediated postprandial hormone–sensitive lipase suppression than Pro12 homozygotes in vivo (25). The Ala12 allele has been shown to be protective against adult type 2 diabetes (5,23,26), but this allele is also associated with increased BMI in overweight individuals in meta-analyses (27). The *PPARG* gene may therefore provide a genetic link between the association of preterm birth and diseases in later life. In accordance with this result, data from Eriksson et al. (28) showed that in Finns, the well-known association existing between small birth weight and insulin resistance later in life was seen only in *Pro12Pro* individuals. The *Pro12Pro* genotype was associated with insulin resistance at the age of 70 only among those who had a birth weight <3,500 g. Unfortunately, no details on gestational duration were available in this study, so we cannot evaluate whether the low birth weight was due to a reduced gestational duration. Other findings have been described in a French sample of 20-year-old individuals among whom the Ala12 allele was associated with higher insulin resistance indexes in SGA individuals (8). In contrast to our present findings, these authors did not find any association between the *PPARG* Pro12Ala polymorphism and SGA. Other large cohorts are required before any definitive conclusions can be drawn about the role and the long-term impact of the *PPARG* Pro12Ala polymorphism on the risk of preterm birth.

Our study has several limitations. First, the number of individuals born before term is limited (60 subjects born before 37 weeks and 24 subjects born before 35 weeks). Nevertheless, despite weak statistical power, the effect of the *PPARG* Pro12Ala polymorphism on preterm birth was consistent for two independent cohorts and when using the two cut-off dates. Second, it remains to be established whether the genetic susceptibility to preterm delivery is primarily a maternal or a child effect, the two being tightly linked because mother and offspring share around 50% of their genetic material. Third, our cohorts lacked records on maternal diseases during pregnancy such as gestational diabetes, a known cause of preterm birth. However, because gestational diabetes is rare (2–5% of pregnant women), it is a condition difficult to study in any epidemiological cohorts of general population samples. Fourth, it would be valuable to distinguish spontaneous preterm births from induced preterm births. Fifth, we could not study the recessive effect of the Pro12Ala polymorphism because the number of homozygote Ala12Ala individuals was too small. Lastly, it is not possible to rule out the hypothesis that the *PPARG* Pro12Ala polymorphism might be related to fetal survival in cases where premature birth occurs rather than preterm birth per se. The observation that the Ala12 allele was more frequent in preterm infants could also be explained by a protective role of the polymorphism against fetal death (if the fetus dies, these individuals would not enter our studies). This hypothesis is supported by the fact that the Ala12 allele frequency is higher in the group of mothers who had previous stillbirths and miscarriages than in the group of mothers who did not have any (Table 5).

In conclusion, we report for the first time that the *PPARG* Pro12Ala polymorphism might be associated with preterm birth in samples from Northern Ireland. Large long-term prospective studies including *1*) genetic material from mothers, children, spontaneous abortions, and very early gestational age babies, and *2*) acute measures of gestational duration as well as detailed maternal data are needed to truly evaluate the impact of the *PPARG* Pro12Ala polymorphism on preterm birth and associated diseases later in life.

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