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# A Functional Polymorphism in a STAT5B Site of the Human *PPAR* $\gamma$ 3 Gene Promoter Affects Height and Lipid Metabolism in a French Population

Aline Meirhaeghe, Lluís Fajas, Fabrice Gouilleux, Dominique Cottel, Nicole Helbecque, Johan Auwerx, Philippe Amouyel

**Objective**—The peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) plays a role in adipocyte differentiation and insulin sensitization. It has been shown that genetic variation in the *PPAR* $\gamma$  gene alters body weight control, lipid and insulin homeostasis, and the susceptibility to type 2 diabetes. Four PPAR $\gamma$  isoforms are generated by alternative splicing and promoter usage. PPAR $\gamma$ 3 is only expressed in adipose tissue, colon, and macrophages and therefore seems to be a good candidate gene for metabolic and cardiovascular-associated diseases. In the present study, we looked for genetic variation in the *PPAR* $\gamma$ 3 promoter.

**Methods and Results**—The proximal *PPAR* $\gamma$ 3 promoter was sequenced in 20 individuals. We detected a C/G polymorphism at position -681 from exon A2. Interestingly, it was located in a signal transducer and activator of transcription 5B (STAT5B) binding consensus site. In a French population (n=836), the -681G allele was associated with increased height and plasma low-density lipoprotein cholesterol concentrations. In vitro, we showed that the -681G allele completely abolished the binding of STAT5B to the cognate promoter element as well as the transactivation of the *PPAR* $\gamma$ 3 promoter by the growth hormone/STAT5B pathway.

**Conclusions**—Our results suggest that PPAR $\gamma$ 3 may regulate the control of height and lipid homeostasis via the STAT5B pathway. (*Arterioscler Thromb Vasc Biol.* 2003;23:289-294.)

**Key Words:** obesity ■ cholesterol ■ PPAR ■ growth hormone ■ STAT

Peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) belongs to the nuclear hormone receptor superfamily and heterodimerises with retinoid X receptor (RXR) to regulate target genes involved in adipocyte differentiation<sup>1,2</sup> and insulin sensitization.<sup>3</sup> PPAR $\gamma$  is activated by several fatty acid derivatives, such as 15-deoxy- $\Delta^{12,14}$ -prostaglandin J<sub>2</sub>, 9- and 13-HODE, and linoleic acid.<sup>4-6</sup> Furthermore, PPAR $\gamma$  is the receptor that mediates the antidiabetic effects of thiazolidinediones,<sup>7</sup> the oral agents used in the treatment of type II diabetes.<sup>8</sup>

PPAR $\gamma$  is implicated in several processes, such as adipose,<sup>9,10</sup> macrophage,<sup>11</sup> and breast<sup>12</sup> and colon cell differentiation,<sup>13</sup> as well as in glucose and lipid homeostasis.<sup>14</sup> The *PPAR* $\gamma$  gene produces 4 different PPAR $\gamma$  mRNAs by alternative splicing and promoter usage,<sup>15-19</sup> giving rise to 2 different proteins, with PPAR $\gamma$ 1, PPAR $\gamma$ 3, and PPAR $\gamma$ 4 being similar and PPAR $\gamma$ 2 having an additional 30 amino acids at the N terminus.<sup>18</sup> PPAR $\gamma$ 1 is ubiquitously expressed, PPAR $\gamma$ 2 is restricted to adipose tissue, and PPAR $\gamma$ 3 seems mainly confined to macrophages, adipose tissue, and colon.<sup>16</sup> The tissue distribution of PPAR $\gamma$ 4 has not been explored yet.<sup>19</sup> Based on this tissue distribution pattern, PPAR $\gamma$ 3 is an interesting candidate gene for

genetic susceptibility to metabolic disorders and associated cardiovascular diseases. Mutations in the PPAR $\gamma$  gene have been of great interest in helping to study and unravel the complex and multiple biological actions of PPAR $\gamma$  in vivo. The following mutations in the PPAR $\gamma$  gene have been described: (1) a silent C/T polymorphism in exon 6 associated with higher plasma leptin levels in obese subjects<sup>20</sup>; (2) a Pro12Ala substitution in *PPAR* $\gamma$ 2 associated with lower PPAR $\gamma$ 2 activity in vitro and decreased type II diabetes risk<sup>21</sup>; (3) a very rare Pro115Gln mutation that renders the protein constitutively active associated with a severe obesity phenotype<sup>22</sup>; and (4) heterozygous mutations in the ligand-binding domain associated with severe insulin resistance.<sup>23</sup> To address the possibility that sequence variations in the promoter of *PPAR* $\gamma$ 3 might be associated with altered expression regulation of *PPAR* $\gamma$ 3, we sequenced the exon A2 and the *PPAR* $\gamma$ 3 promoter in 20 subjects coming from the Northern France MONICA population study.

## Methods

The Methods section is available online at <http://atvb.ahajournals.org>.

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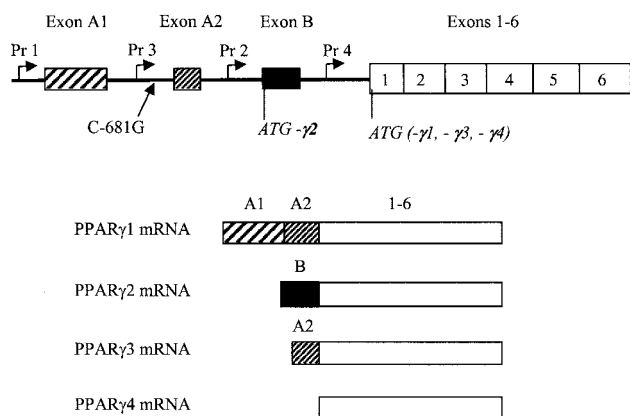
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**Figure 1.** Promoter organization of the human *PPAR $\gamma$*  gene. A scheme of the genomic structure of the human *PPAR $\gamma$*  gene and the various transcripts. Pr stands for promoter. Exons 1 to 6 are common to all isoforms. *PPAR $\gamma$ 1* contains the untranslated exons A1 and A2, *PPAR $\gamma$ 3* the untranslated exon A2. *PPAR $\gamma$ 2* contains the translated exon B (30 amino acids). The *PPAR $\gamma$ 1*,  $-\gamma$ 3, and  $-\gamma$ 4 proteins are identical and contain exons 1 to 6. The position of the polymorphism is indicated by an arrow.

## Results

A fragment of 851 bp (777 bp of the *PPAR $\gamma$ 3* promoter and 74 bp of the exon A2) (Figure 1) was sequenced in 20 French subjects. Two polymorphisms were detected: a T/C polymorphism at position  $-553$  and a C/G substitution at position  $-681$  from the beginning of exon A2 (GenBank accession No. AF548352). These two polymorphisms were in complete linkage disequilibrium, but only the C-681G polymorphism was located in the sequence motif TTCATGGAA (polymorphism underlined) that closely resembles the  $\gamma$ -interferon-activated sequence (GAS)-like element (GLE) TTCNNGAA. This element has been demonstrated to be a DNA-binding site for transcription factors of the signal transducer and activator of transcription (STAT) family.<sup>24,25</sup> A polymorphism in this motif might therefore have a functional impact. Consequently, we decided to analyze the C-681G polymorphism in our French population. The genotype distribution of the *PPAR $\gamma$ 3* C-681G polymorphism ( $n=836$ ) was as follows: CC, 56.6%; CG, 36.9%; and GG, 6.5% (G allele frequency, 0.25) (Table 1). This distribution was in Hardy-Weinberg equilibrium. No differences were observed between genders. These frequencies were similar in subjects with type II diabetes ( $n=49$ ) or with obesity ( $n=232$ ) (subjects defined with a body mass index [BMI]  $\geq 30$  kg/m<sup>2</sup>) (data not shown). We explored the potential relationship between the *PPAR $\gamma$ 3* C-681G polymorphism and obesity markers such as body weight, body mass index,

plasma leptin levels, and plasma glucose and lipid-related variables. Subjects carrying the  $-681$  G allele were taller and had higher body weight, plasma total and low-density lipoprotein (LDL)-cholesterol, and apolipoprotein B concentrations than CC subjects (Table 2). Because medication during adulthood would not have an effect on height, the impact of the polymorphism on height was also analyzed in the entire population ( $n=1133$ ), and similar results were found (data not shown). No association with body mass index, waist to hip ratio, or plasma leptin levels could be detected. These results were consistent in both sexes (data not shown).

The C-681G polymorphism is located in a putative STAT binding site. The members of the STAT family are latent cytoplasmic transcription factors that after being tyrosine-phosphorylated in response to various hormones and cytokines (eg, prolactin, growth hormone, interferon  $\gamma$ , and tumor necrosis factor [TNF]- $\alpha$ ) translocate to the nucleus, where they regulate the transcription of target genes. We first evaluated the ability of STAT3, STAT5A, and STAT5B to transactivate the *PPAR $\gamma$ 3* promoter. We created a vector containing the *PPAR $\gamma$ 3* promoter with the C allele in front of the luciferase gene (pGL3 $\gamma$ 3/C). This reporter vector was cotransfected in mouse preadipocyte 3T3-L1 cells together with expression vectors encoding the growth hormone receptor (GHR) and either STAT3, or STAT5A or STAT5B. Cells were incubated in the absence or presence of human growth hormone (hGH), an activator of the STAT proteins. Cells cotransfected with activated STAT5B showed a 3-fold induction of *PPAR $\gamma$ 3* promoter activity compared with nonactivated STAT5B transfected cells (Figure 2A). Neither activated STAT5A nor activated STAT3 had an effect on the activity of the *PPAR $\gamma$ 3* promoter (Figure 2A), suggesting that the expression of *PPAR $\gamma$ 3* seems specifically regulated by STAT5B. To confirm that the activity of the *PPAR $\gamma$ 3* promoter was specific of STAT5B, a vector expressing the transactivation domain-truncated version of STAT5B (STAT5B $\Delta$ 754) that acts as a dominant negative toward the full-length STAT5B<sup>26</sup> was cotransfected with the *PPAR $\gamma$ 3*-luciferase reporter construct and cells were stimulated by hGH. No induction of the *PPAR $\gamma$ 3* promoter activity could be detected with the dominant-negative STAT5B vector (Figure 2B). This experiment showed that the *PPAR $\gamma$ 3* transactivation was specific of STAT5B and that the transactivation domain of STAT5B was necessary to mediate this effect.

To explore whether the *PPAR $\gamma$ 3* C-681G polymorphism had any influence on the regulation of the *PPAR $\gamma$ 3* promoter by STAT5B, we made a *PPAR $\gamma$ 3*-luciferase reporter vector including the G allele (pGL3 $\gamma$ 3/G). 3T3-L1 cells cotrans-

**TABLE 1. Genotype and Allele Frequencies of the *PPAR $\gamma$ 3* C-681G Polymorphism**

	n	Genotype			Allele	
		CC	CG	GG	C	G
All	836	473 (56.6)	309 (36.9)	54 (6.5)	1255 (0.75)	417 (0.25)
Men	419	229 (54.7)	164 (39.1)	26 (6.2)	622 (0.74)	216 (0.26)
Women	417	244 (58.5)	145 (34.8)	28 (6.7)	633 (0.76)	201 (0.24)

Data are n (%) for genotypes and n (frequency) for alleles.

**TABLE 2.** Influence of the *PPAR $\gamma$ 3* C-681G Polymorphism on Anthropometric and Biological Variables

Genotype	CC (n=473)	CG (n=309)	GG (n=54)	P (3 classes)	P (CC vs CG+ GG)
Clinical variables					
Height, cm*	166.9±9.4	168.4±9.3	168.1±8.8	0.08	<b>0.03</b>
Weight, kg*	71.2±14.5	73.6±14.8	74.5±15.3	<b>0.02</b>	<b>0.008</b>
BMI, kg/m <sup>2</sup> *	25.5±4.5	25.8±4.4	26.2±4.2	NS	NS
Waist, cm*	87.9±13.0	88.8±12.8	90.0±11.9	NS	NS
Waist-to-hip ratio*	0.87±0.09	0.87±0.09	0.87±0.08	NS	NS
Biological variables					
Leptin, ng/mL*	10.3 (4.0–26.3)	10.2 (4.2–24.5)	11.2 (4.4–28.5)	NS	NS
Total cholesterol, mmol/L†	5.74±1.03	5.95±1.07	6.07±1.05	<b>0.01</b>	<b>0.003</b>
LDL cholesterol, mmol/L†	3.62±0.99	3.87±1.06	3.96±0.98	<b>0.004</b>	<b>0.001</b>
Apolipoprotein B, g/L†	1.15±0.28	1.22±0.31	1.23±0.27	<b>0.009</b>	<b>0.002</b>
HDL cholesterol, mmol/L†	1.54±0.48	1.50±0.47	1.56±0.52	NS	NS
Triglycerides, mmol/L†	1.07 (0.62–1.84)	1.15 (0.62–2.12)	1.04 (0.62–1.73)	NS	NS
Insulin, $\mu$ U/mL†	12.4 (7.2–21.3)	12.8 (7.8–21.1)	12.2 (7.5–19.7)	NS	NS
Glucose, mmol/L†	5.15 (4.53–5.87)	5.21 (4.53–5.99)	5.37 (4.66–6.17)	NS	NS

Data are means±SD. \*P values were adjusted for age, sex, insulin levels, and alcohol and cigarette consumptions. †P values were adjusted for age, sex, BMI, and alcohol and cigarette consumptions.

ected with either the pGL3 $\gamma$ 3/G or pGL3 $\gamma$ 3/C vector and with expression vectors encoding GHR and STAT5B were incubated in the absence or presence of hGH. Cells stimulated with hGH transfected with pGL3 $\gamma$ 3/G showed lower *PPAR $\gamma$ 3* promoter activity than cells transfected with pGL3 $\gamma$ 3/C at levels comparable to the empty pGL3 vector (Figure 2C).

To additionally investigate the specific transactivation of the *PPAR $\gamma$ 3* promoter by STAT5B, we evaluated the ability of STAT5A and STAT5B to bind to the *PPAR $\gamma$ 3* promoter. Nuclear extracts from COS7 cells cotransfected with either STAT5A or STAT5B and GHR expression vectors, stimulated with hGH or not, were prepared and used for electrophoretic mobility shift assays (Figure 3). Radiolabeled 20-mer oligonucleotides containing either the *PPAR $\gamma$ 3* STAT C or G allele were used as probes. We used the high-affinity STAT binding site from the  $\beta$ -casein gene promoter as a positive control. As expected, no bandshift was detected with nuclear extracts from cells unstimulated with hGH, consistent with the presence of nonactive STAT proteins (lanes 1, 3, 5, 7, 9, and 11). When nuclear extracts from hGH-treated cells transfected with STAT5B were used, a binding to the STAT site containing the C allele could be observed (lane 4). Similar protein-DNA complex was observed when the STAT-binding site of the  $\beta$ -casein was used as a positive probe, suggesting that this DNA-protein complex probably contains the STAT5 protein (lanes 10 and 12). Conversely, STAT5A did not bind to the C allele-containing STAT site (lane 2). When the oligonucleotide containing the G allele was used as a probe with nuclear extracts from cells transfected with activated STAT5B, no binding could be observed (lane 8).

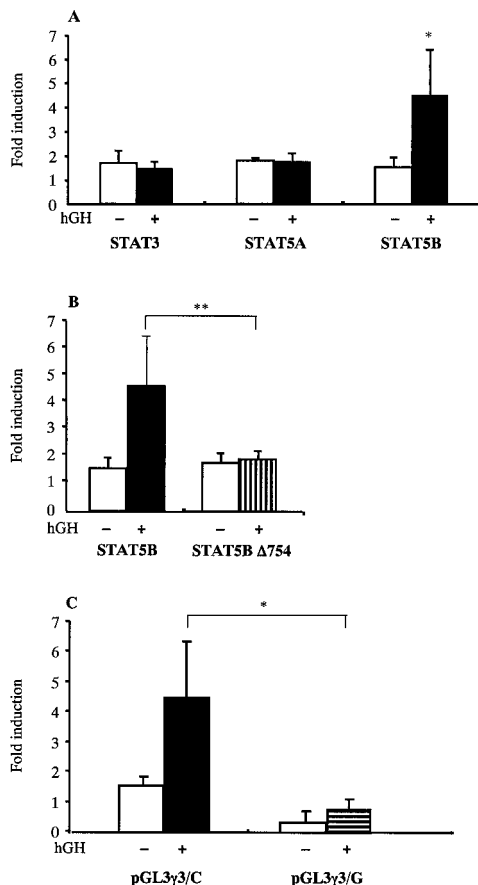
## Discussion

Taken together, our results suggest that a biallelic polymorphism in the transcriptional regulatory region of *PPAR $\gamma$ 3*

influences height and lipid homeostasis in humans, maybe through the modulation of the *PPAR $\gamma$ 3* gene transcription by the STAT5B pathway.

We demonstrated that the GH/STAT5B pathway could activate the *PPAR $\gamma$ 3* promoter in 3T3-L1 cells and that the *PPAR $\gamma$ 3* C-681G polymorphism prevented it. Furthermore, STAT5B was able to bind the *PPAR $\gamma$ 3* STAT consensus site, whereas STAT5A was not. STAT5A and STAT5B proteins are products of two different genes and show an  $\approx$ 90% amino acid similarity.<sup>27</sup> STAT5A and STAT5B have both the abilities of binding the prolactin response element of the  $\beta$ -casein gene<sup>27</sup> and of being activated by GH. However, they differ most markedly in their DNA-binding specificity,<sup>28</sup> which may explain the specific *PPAR $\gamma$ 3* transactivation by STAT5B only. We also showed that the C-681G allelic variation completely abolished the binding of STAT5B to the *PPAR $\gamma$ 3* promoter, which could explain the lower activity of the mutant *PPAR $\gamma$ 3* promoter. We are not excluding the fact that other components than GH such as prolactin, interleukins, erythropoietin, or TNF- $\alpha$  can also activate the STATs and modulate *PPAR $\gamma$ 3* expression. Indeed, it has been shown previously that TPA stimulates *PPAR $\gamma$ 3* expression in macrophages<sup>17</sup> or that prolactin enhances *PPAR $\gamma$ 3* expression in NIH-3T3 cells.<sup>29</sup> Similarly, a STAT1 binding site has been described in the *PPAR $\gamma$ 2* promoter, and IFN- $\gamma$  could decrease the promoter activity by acting through this STAT1 site.<sup>30</sup> Therefore, the STAT proteins might have an important role in *PPAR $\gamma$ 3* expression regulation.

The higher height in G allele bearers suggests that *PPAR $\gamma$ 3* might be implicated in the growth of the skeleton. Several studies have now shown a role for *PPAR $\gamma$ 3* in osteoblast/osteoclast activity. It has been shown that low doses of *PPAR $\gamma$ 3* ligands induce the osteoblastic maturation of mouse preosteoblast cells, even though high doses inhibit it.<sup>31</sup> Lecka-Czernik and colleagues<sup>32,33</sup> demonstrated that activa-



**Figure 2.** Analysis of the *PPAR* $\gamma$ 3 promoter activity in transient transfection assays. **A**, Effect of STATs proteins on *PPAR* $\gamma$ 3 promoter activity. 3T3-L1 cells were transiently cotransfected with either empty pGL3 or pGL3 $\gamma$ 3/C, STAT3, STAT5A or STAT5B, and GHR expression vectors. Activity of pGL3 $\gamma$ 3 is expressed as fold activity compared with the activity of empty pGL3. Cells were stimulated (black bars) or not (white bars) with 500 ng/mL hGH. Each column represents the mean  $\pm$  SD of 5 independent experiments. Luciferase activities were normalized to the  $\beta$ -galactosidase activities. \*hGH stimulated versus unstimulated,  $P=0.02$  (Kruskal-Wallis test). **B**, Study of a dominant-negative STAT5B expression construct. 3T3-L1 cells were transiently cotransfected with either empty pGL3 or pGL3 $\gamma$ 3/C, STAT5B (black bars) or STAT5B  $\Delta$ 754 (vertically hatched bars), and GHR expression vectors. Activity of pGL3 $\gamma$ 3/C when cells were stimulated with 500 ng/mL hGH expressed as fold activity compared with the activity of empty pGL3 is represented. Each column represents the mean  $\pm$  SD of 5 independent experiments. Luciferase activities were normalized to the  $\beta$ -galactosidase activities. \*\*STAT5B versus STAT5B  $\Delta$ 754,  $P=0.004$  (Kruskal-Wallis test). **C**, Comparison of *PPAR* $\gamma$ 3 promoter activities according to the C or G allele. 3T3-L1 cells were transiently cotransfected with empty pGL3 or pGL3 $\gamma$ 3/C or pGL3 $\gamma$ 3/G, STAT5B and GHR expression vectors. Activity is expressed as fold activity compared with the activity of empty pGL3. Cells were stimulated or not with 500 ng/mL hGH. Each column represents the mean  $\pm$  SD of 5 independent experiments. Luciferase activities were normalized to the  $\beta$ -galactosidase activities. \*pGL3 $\gamma$ 3/C versus pGL3 $\gamma$ 3/G,  $P=0.01$  (Kruskal-Wallis test).

tion of *PPAR* $\gamma$ 2 with rosiglitazone in U-33/ $\gamma$ 2 cells stimulated the differentiation of the cells into adipocytes and blocked their ability to become osteoblasts. The activation of the *PPAR* $\gamma$  pathway also inhibits the osteoclast formation and activation.<sup>34,35</sup> Moreover, Ogawa et al<sup>36</sup> reported that primary osteoblasts express *PPAR* $\gamma$  and that the common *PPAR* $\gamma$  C/T

exon 6 polymorphism is associated with lower bone mineral density in Japanese postmenopausal women. Our results additionally support a potential role for *PPAR* $\gamma$  in bone metabolism. Growth hormone (GH) is the key hormone of bone metabolism. Its effects are mediated through activation of the GHR,<sup>37</sup> the receptor-associated tyrosine kinase JAK2,<sup>38</sup> and the STATs.<sup>25,40</sup> *PPAR* $\gamma$  may modulate some of the effects of GH. A potential modulatory effect of *PPAR* $\gamma$  over the STAT signaling pathway is suggested by Ricote et al,<sup>11</sup> who showed that the natural or synthetic *PPAR* $\gamma$  ligands can inhibit STAT activity in a *PPAR* $\gamma$ -dependent manner. Therefore, *PPAR* $\gamma$  could attenuate STAT signaling and potentially interfere with GH effects. As a consequence of the C-681G polymorphism, the expression of *PPAR* $\gamma$  would be decreased in response to GH, and if we assume that *PPAR* $\gamma$  inhibits STAT5B, which is a GH mediator, then a mutation in the STAT site is equivalent to increased levels of GH, resulting in a possible increase in final height and body weight of subjects bearing the -681 G allele. The absence of effects of the *PPAR* $\gamma$ 3 C-681G polymorphism on BMI, waist to hip ratio, or plasma leptin levels suggests that the increased body weight in G allele bearers was attributable to increased lean body mass rather than increased fat mass.

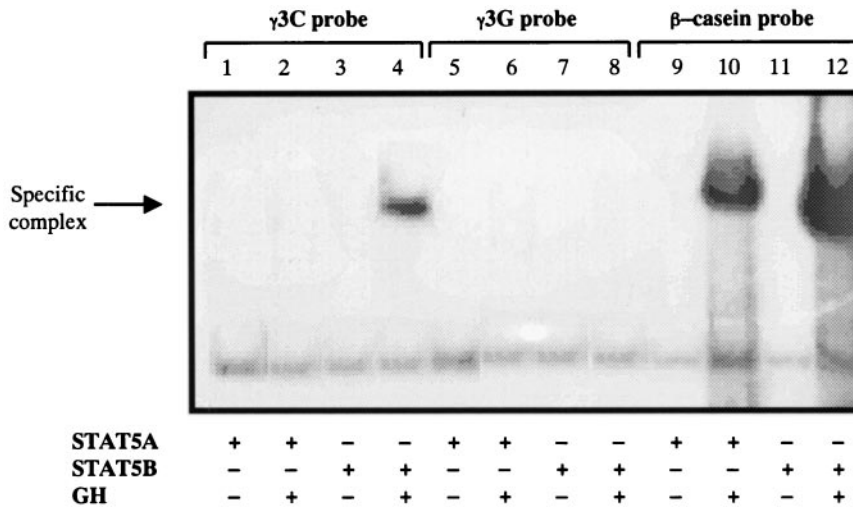
Moreover, subjects carrying the G allele had increased plasma apolipoprotein B and total and LDL-cholesterol levels, important cardiovascular risk factors. Monocytes and macrophages, where *PPAR* $\gamma$ 3 is expressed, are pivotal to the development of atherosclerosis. Ricote et al<sup>11</sup> showed that *PPAR* $\gamma$  inhibits the expression of inducible nitric oxide synthase, gelatinase B, and the scavenger receptor A genes in response to synthetic ligands in macrophages, probably by antagonizing the transcription factors AT-1, STAT, and nuclear factor- $\kappa$ B. Moreover, our results can also be connected to the fact that *PPAR* $\gamma$  is an important regulator of the scavenger receptor CD36, a receptor that is thought to be important during macrophage uptake of modified LDL and foam cell formation.<sup>41</sup>

Expression of STAT5A and STAT5B are elevated in murine and human adipocytes compared with their fibroblast precursors.<sup>42,43</sup> Moreover, the induction of these proteins is regulated in a *PPAR* $\gamma$  ligand-dependent manner during adipogenesis.<sup>44</sup> The fact that STAT5B knockout mice display elevated plasma GH, abnormal growth rate, and impaired adipocyte differentiation (no lipids in the cells)<sup>45,46</sup> reinforces the involvement of STAT5B as a mediator of GH and as a factor important in adipocyte differentiation. Therefore, the STAT proteins may regulate fat-specific gene expression as well as upstream transcriptional factors like *PPAR* $\gamma$  and be themselves regulated by those transcriptional factors.

In conclusion, our data suggest that *PPAR* $\gamma$ 3 may be involved in the control of height and lipid homeostasis in humans through the GH/STAT5B pathway and may mediate some of the proatherogenic effects of GH.

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**Figure 3.** Electrophoretic mobility shift assay. Radiolabeled PPAR $\gamma$  C allele (lanes 1 through 4) or PPAR $\gamma$  G allele (lanes 5 through 8) or  $\beta$ -casein (lanes 9 through 12) probes were incubated with nuclear extracts prepared from COS7 cells either unstimulated (lanes 1, 3, 5, 7, 9, and 11) or stimulated with hGH (1  $\mu$ g/mL) (lanes 2, 4, 6, 8, 10, and 12). Cells were transfected with STAT 5A (lanes 1, 2, 5, 6, 9, and 10) or STAT5B (lanes 3, 4, 7, 8, 11, 12). Arrow indicates the migration of the GH-induced DNA-protein complexes.

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