Review

Retinoid X Receptor Heterodimer Variants and Cardiovascular Risk Factors

Atsushi Nohara, Junji Kobayashi, and Hiroshi Mabuchi

Departments of Lipidology, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan

Nuclear receptors are transcription factors that can be activated by specific ligands. Recent progress has shown that retinoid X receptor (RXR) and its heterodimerization partners, including peroxisome proliferator-activated receptors, regulate many important genes involved in energy homeostasis and atherosclerosis, and should be promising therapeutic targets of metabolic syndrome. RXR heterodimers regulate a number of complex cellular processes, and genetic studies of RXR heterodimers have provided important clinical information in addition to knowledge gained from basic research. Genetic variants of RXR heterodimers were screened and investigated, and some variants were shown to have a considerable impact on metabolic disorders, including phenotypic components of familial combined hyperlipidemia. The combined efforts of basic and clinical science regarding nuclear receptors have achieved significant progress in unraveling the inextricably linked control system of energy expenditure, lipid and glucose homeostasis, inflammation, and atherosclerosis. This review summarizes the current understanding regarding RXR heterodimers based on their human genetic variants, which will provide new clues to uncover the background of multifactorial disease, such as metabolic syndrome or familial combined hyperlipidemia.


Key words; Nuclear receptors, Gene mutations, Lipoprotein lipase, Familial combined hyperlipidemia, Metabolic syndrome

Introduction

Nuclear receptors contain a DNA-binding domain and a ligand-binding domain, and are activated by specific ligands to regulate target gene transcription. Clinically available lipid-lowering fibrates are activators of peroxisome proliferator-activated receptor alpha (PPARα), and insulin-sensitizing thiazolidinediones (TZD) are activators of PPARγ. These molecules are key regulators of metabolism and inflammation, and both play critical roles in the development of cardiovascular disease. Many nuclear receptors, especially retinoid X receptor (RXR) and its heterodimerization partners, play important roles in metabolic homeostasis. RXR heterodimers bind to a variety of ligands derived from cholesterol, fatty acids, and glucose to regulate target genes that mediate metabolic homeostasis. Recent studies indicated that not only PPARs but also other RXR heterodimers are attractive therapeutic targets for intervention in atherosclerosis.

Metabolic regulation of RXR heterodimers is complex, involving a great deal of cross-talk, and their clinical importance has been clarified through investigations of genetic variants. Familial combined hyperlipidemia (FCHL) is the most common form of hereditary hyperlipidemia, but its genetic background has not been fully elucidated. Increased in very-low density lipoprotein (VLDL) with or without that in low-density lipoprotein (LDL) is the main characteristic of patients with FCHL. The lipoprotein lipase (LPL) gene has been extensively studied due to its pivotal role in the hydrolysis of VLDL. Genetic variants of LPL have been reported to contribute to the lipid phenotype in FCHL, but LPL variants themselves were not linked to the FCHL phenotype. At present,
LPL is recognized as a modifier gene of the FCHL phenotype. "Intra-individual variability" of the lipoprotein phenotype is often included as a criterion in diagnosis; however, a recent prospective study of FCHL families suggests that this variability may even include normolipidemic periods in affected subjects. This feature indicates that FCHL could be a "disease of regulation" rather than a genetic defect in certain peripheral components of lipid metabolism. As RXR heterodimers play pivotal roles in lipid metabolism, we screened all coding regions of PPARα, PPARγ2, PPARδ, farnesoid X receptor (FXR), liver X receptor alpha (LXRα) and RXRγ genes in hyperlipidemic patients, including FCHL. We found that the RXRγ gene, located on the so-called "FCHL locus" on chromosome 1q21-q23, could contribute to the genetic background of FCHL.

This review summarizes the current understanding regarding RXR heterodimers, including PPARs, based on their human genetic variants (Table 1).

**RXRs**

RXRs are the major heterodimerization partners of the nuclear receptors mentioned below, and have three subtypes, RXRα, RXRβ, and RXRγ. No rexinoid (RXR-selective retinoid) with apparent subtype selectivity has been identified so far. RXRα is predominantly expressed in the liver, kidney, epidermis, and intestine. RXRβ is ubiquitously expressed. RXRγ is expressed mostly in muscle, some parts of the brain, and very weakly in adipocytes with enhancement by TZD. RXR partners do not exhibit a marked preference for one of the three RXR subtypes in most cases. Drugs targeting heterodimerization partners of RXRs are already in clinical use for the treatment of cancer, endocrine disorders, dermatological diseases, and metabolic syndrome.

Despite numerous studies since their initial discovery, RXRs remain enigmatic nuclear receptors, and there is still no consensus regarding their role. RXR heterodimer partners can be classified into functionally distinct "permissive" and "non-permissive" groups. PPARs, LXRα and FXR form permisive RXR heterodimers, which synergistically respond to both agonists of RXR and the partner receptor. Retinoic acid receptors (RARs), thyroid hormone receptor, and vitamin D receptor form non-permissive RXR heterodimers, which cannot be activated by an RXR agonist but only by the agonist of the partner receptor. Presumably, non-permissive partners inhibit RXR activation. RXRs can also form functional homodimers. Because few RXR-specific target genes have been identified, the importance of RXR homodimers in vivo has not been well established, but at least RXR homodimers can activate PPAR target genes in vivo. These findings were established mainly by the RXRα gene, and limited information is available about the RXRγ gene. Genetic or pharmacological modification of RXRs would result in changed RXR heterodimer function, also depending on its counterparters, and furthermore, in changed RXR homodimer function, and this would be more complex with crosstalk than the modification of partner receptors.

It is known that retinoids, which activate both RARs and RXRs, induce hypertriglyceridemia through decreased clearance of VLDL by an LPL-dependent pathway; however, the effects of rexinoids in lipid metabolism are less clear. Synthetic RXR ligands induced hypertriglyceridemia through decreased clearance of VLDL by LPL-dependent pathways, except in one study. Recent studies suggest that different RXR agonists do not necessarily display the same biological effects, and can target specific heterodimer partners.

In contrast to the high rates of embryonic lethality of RXRα- and RXRβ-deficient mice, RXRγ-deficient mice are apparently normal, but have been reported to have reduced fasting plasma TG levels, increased skeletal muscle LPL activity, and less body weight gain due to low fat mass only when fed a high-fat diet.

**Polymorphisms in RXRα Gene and RXRβ Gene**

Several polymorphisms have been reported in the RXRα gene, but their associations with metabolic disorders have not been reported. Obesity and gallstones, both related to insulin resistance, are linked to an elevated risk of biliary cancer. Recently, Chang et al., a group in the National Institutes of Health of United States, reported that RXRβ c.51C>T polymorphism is associated with a higher body mass index (BMI), higher risk of gallstones, and elevated risk of bile duct cancer. Functional consequence of this SNP was not confirmed.

**RXRγ Gene p.Gly14Ser**

In humans, some RXRγ gene variants have been reported to be associated with free fatty acid levels and TG levels in familial type 2 diabetes, but none of the variants showed an altered coding sequence. The human RXRγ gene is located in chromosome 1q21-q23, the so-called "FCHL locus," and linkage analysis and twin study indicated that the RXRγ gene was also linked with higher LDL-cholesterol and TG in Chinese and German families.
<table>
<thead>
<tr>
<th>RXR allele</th>
<th>Molecular mechanism</th>
<th>Clinical phenotype of rare allele</th>
<th>Ethnicity, species or countries [References]</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.Val227Ala</td>
<td>Reduced function</td>
<td>BMI↑, TG↑, non-alcoholic fatty liver disease↑, waist circumferenc↑</td>
<td>Japanese [55], Chinese [55, 56]</td>
</tr>
<tr>
<td>SNP6</td>
<td>DM↑, association with FFA</td>
<td>DM↑, association with FFA, TG and HOMA-IR</td>
<td>North European [20]</td>
</tr>
<tr>
<td>SNP11</td>
<td>DM↑, association with FFA</td>
<td>DM↑, association with FFA and TG</td>
<td>North European [20, 24]</td>
</tr>
</tbody>
</table>

**Table 1. RXR Variants and Associated Metabolic Phenotypes**

<table>
<thead>
<tr>
<th>RXR allele</th>
<th>Molecular mechanism</th>
<th>Clinical phenotype of rare allele</th>
<th>Ethnicity, species or countries [References]</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.Leu162Val</td>
<td>Gain of function (reduced at baseline)</td>
<td>LDL-C↑, apoB↑, TG↑, DM↑, obesity↑ [44, 47]↑, aththerosclerosis↑</td>
<td>Caucasian [41, 42, 43, 48, 49], African-American [43], French-Canadian [44], Germany [66], multi-national study [47]</td>
</tr>
<tr>
<td>p.Val227Ala</td>
<td>Reduced function</td>
<td>TC↓, TG↑, non-alcoholic fatty liver disease↓, waist circumferenc↓</td>
<td>Japanese [54], Chinese [55, 56]</td>
</tr>
<tr>
<td>PPAR5</td>
<td>Reduced promoter activity</td>
<td>insulin resistance↑, partial lipodystrophy↑, insulin resistance↑↑↑, lipodyopthy↑, TG↑, DM↑↑↑</td>
<td>not described [107]</td>
</tr>
<tr>
<td>PPAR6</td>
<td>Reduced promoter activity</td>
<td>insulin resistance↑, partial lipodystrophy↑, metabolic syndrome↑</td>
<td>North European [106]</td>
</tr>
<tr>
<td>c.-87T&gt;C (+294T&gt;C)</td>
<td>Gain of function</td>
<td>LDL-C↑, HDL-C↓, FPG↑, BMI↑</td>
<td>metabolic syndrome↑</td>
</tr>
<tr>
<td>hsl</td>
<td>—</td>
<td>BMI↑</td>
<td>Finnish [123]</td>
</tr>
<tr>
<td>bg1</td>
<td>—</td>
<td>systolic BP↑, LDL-C↑, skeletal muscle glucose uptake↑</td>
<td>Changes in adiposity, steatosis, and relative muscle mass with lifestyle intervention [124]</td>
</tr>
<tr>
<td>n503409, n6902123, rs2267668</td>
<td>—</td>
<td>BMI↑</td>
<td>Finnish [124]</td>
</tr>
</tbody>
</table>

**LXK**

<table>
<thead>
<tr>
<th>RXR allele</th>
<th>Molecular mechanism</th>
<th>Clinical phenotype of rare allele</th>
<th>Ethnicity, species or countries [References]</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.-6G&gt;C</td>
<td>Reduced function</td>
<td>TC↑</td>
<td>French-Canadian [135]</td>
</tr>
<tr>
<td>c.-4A&gt;G</td>
<td>Reduced function</td>
<td>TC↑</td>
<td>French-Canadian [135]</td>
</tr>
<tr>
<td>c.-229238</td>
<td>Female obesity↑</td>
<td>life span↑, TG↑, apoE↑</td>
<td>The Netherlands [137]</td>
</tr>
<tr>
<td>haploinsu2c</td>
<td>—</td>
<td>obesity↑</td>
<td>Sweden [136]</td>
</tr>
<tr>
<td>n2859121</td>
<td>—</td>
<td>obesity↑</td>
<td>Sweden [136]</td>
</tr>
<tr>
<td>c.-1G&gt;T</td>
<td>Reduced transcription</td>
<td>BMI↑</td>
<td>Japanese [11]</td>
</tr>
</tbody>
</table>
We identified p.Gly14Ser in the RXRγ gene through mutation screening of RXR-heterodimers in hyperlipidemic patients, and the results indicated that RXRγ Ser14 variant was significantly more frequent in FCHL patients than in other forms of primary hyperlipidemia or the general population\(^\text{11}\). To establish the impact of the identified RXRγ variant on metabolic parameters and coronary atherosclerosis, anthropometric parameters and laboratory data from 175 patients with suspected coronary disease were evaluated. In their lipid profiles, RXRγ Ser14 carriers had higher TG, higher remnant-like lipoprotein particle cholesterol, lower high-density lipoprotein cholesterol (HDL-C), especially in the HDL2 subfraction, and lower apolipoprotein AII levels. RXRγ Ser14 carriers showed a significantly higher coronary stenosis index than those with the wild-type allele\(^\text{11}\).

Subjects with this variant also showed significantly lower LPL activities and protein levels in post-heparin plasma; therefore, transfection assays were performed using the LPL promoter co-transfected with either wild-type RXRγ or the variant. Interestingly, RXRγ Gly14 significantly repressed (~40\%) LPL promoter activity, whereas RXRγ Ser14 showed even stronger repression (~60\%, \(p<0.001\))\(^\text{11}\). These results indicated that RXRγ down-regulates human LPL gene expression, at least partially, by a transcriptional mechanism, and that the newly identified RXRγ variant is a more potent repressor than the wild-type allele. These results suggest that variants of the RXRγ gene contribute to genetic dyslipidemia, including FCHL.

**Other RXRγ Gene Polymorphisms**

Among 14 variants identified in the RXRγ gene, 3 variants (SNP6, SNP11 and SNP13[rs10918169]) in strong linkage disequilibrium showed nominal association with type 2 diabetes mellitus in a case-control study\(^\text{20}\). They also found that these SNPs were associated with free fatty acids, TG and HOMA-IR.

Type 2 diabetes has been linked to chromosome 1q21-24 in multiple samples, including a Utah family sample. Hasstedt et al., a group from Utah University, reported that genotypes of 5 variant pairs, including RXRγ 3’-UTR SNP (rs10918169), accounted for 25.8\% of the genetic variance in type 2 diabetes in these pedigrees\(^\text{24}\). Functional changes of these SNPs were not described.

**PPARs**

The roles of the PPAR family, PPARα, PPARβ/δ, and PPARγ, have been the subject of intensive investigation, because synthetic ligands of PPARα and PPARγ are clinically available as therapeutic drugs. PPARs accept a relatively widerange of compounds as their agonists, which may be because of their large ligand-binding cavity\(^\text{25}\). PPARs have key roles as regulators of energy homeostasis and inflammation, and many synthetic ligands have been developed targeting the ment of treat metabolic disorders. PPARs are known to be expressed in several cancers, and even though the roles of PPARα and PPARδ in carcinogenesis have not been confirmed, TZD is thought to be able to prevent the development of some cancers.

Recent studies have also revealed various unexpected roles of PPARs in cardiovascular homeostasis, as described below.

**PPARα**

PPARα-activating fibrates have been used in the treatment of dyslipidemia for a long time. Among fibrates, fenofibrate is known to be a relatively specific PPARα agonist, and bezafibrate is considered a pan-agonist of PPARs\(^\text{20}\). Bezafibrate showed cardiovascular event reduction in clinical trials, i.e., the Bezafibrate Coronary Atherosclerosis Intervention Trial (BECAIT) and BezaFibrate Infarction Prevention (BIP)\(^\text{27, 28}\) and fenofibrate also showed coronary risk reduction in the Diabetes Atherosclerosis Intervention Study (DAIS) and Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial (no differences in the vprimary end point in the FIELD trial)\(^\text{29, 30}\).

Lipid-lowering fibrates activate PPARα, which is expressed mainly in the liver and regulates many genes involved in the transport and β-oxidation of free fatty acids\(^\text{31}\). PPARα enhances the “reverse cholesterol transport” pathway by up-regulation of not only apolipoprotein AI but also ABCA1\(^\text{32}\).

PPARα is also expressed in the heart and muscle, where it plays a crucial role in controlling fatty acid oxidation. In the heart, PPARα seems to participate in mitochondrial biogenic response and hypertrophic transformation of cardiac myocytes. PPARα regulates the genes responsible for myocardial fatty acid oxidation and is downregulated during cardiac hypertrophy, concomitant with the switch from fatty acid to glucose utilization\(^\text{33}\). Animal studies have shown that PPARα activation prevents cardiac hypertrophy. A recent study demonstrated the induction of neo-angiogenesis by selective activation of PPARα and PPARγ by a vascular endothelial growth factor-dependent mechanism\(^\text{34}\).

PPARα expression is also found in vascular cells, including endothelial cells, vascular smooth muscle cells, and monocytes/macrophages. PPARα is also a
key regulator of inflammatory response, which is a critical process in atherosclerosis\textsuperscript{39}. PPAR\(\alpha\) activation can inhibit inflammatory response genes by repressing nuclear factor-\(\kappa\)B, a signal transducer and activator of transcription, and activator protein-1 signaling. PPAR\(\alpha\) activator inhibits interleukin (IL)-1-induced production of IL-6 and prostaglandin and the expression of cyclooxygenase-2 in vascular smooth muscle cells\textsuperscript{35}. PPAR\(\alpha\) activation reduces vascular cell adhesion molecule-1 expression and increases NO expression and release in vascular cells, maintaining normal endothelial function. Thus, we expect that PPAR\(\alpha\) activation has potential cardio-protective effects in addition to the mere amelioration of dyslipidemia\textsuperscript{36, 37}. PPAR\(\alpha\)’s important role in macrophages is well established. Unexpectedly, we found that PPAR\(\alpha\) induces NADPH oxidase activity in macrophages and generate endogenous PPAR\(\alpha\) ligands through LDL oxidation\textsuperscript{38}. More recently, liver-specific PPAR\(\alpha\) was shown to control the acute-phase response\textsuperscript{39}. Cross-talk between pleiotropic effects of statins and fibrates has also been examined\textsuperscript{40}.

**PPAR\(\alpha\) p.Leu162Val**

A p.Leu162Val polymorphism of the PPAR\(\alpha\) gene has been widely reported in Europe and North American populations\textsuperscript{41}. We screened the p.Leu162Val variant in the Japanese population, detecting no single individual with this variant\textsuperscript{11}. Val162 was shown to be associated with increased LDL-C in the Framingham offspring study\textsuperscript{42}, and with increased apolipoprotein B or TG in other studies\textsuperscript{43, 44}, but the clinical features, especially with regard to obesity and diabetic risk are still controversial\textsuperscript{44-46}. A recent report from the Study to Prevent Non-Insulin-Dependent Diabetes Mellitus (STOP-NIDDM) showed that Val162 increased the risk of diabetes by 1.9-fold and was associated with increased plasma glucose and insulin lev-

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**Fig. 1.** The major actions of RXR heterodimer partners in glucose and lipid metabolism.

RXR heterodimers regulate metabolic organ systems. Dysfunction of these systems contributes to cardiovascular risk factors, including metabolic syndrome.
els\(^{47}\). Val162 is a gain-of-function variant, and the results of in vitro and animal studies suggested that this variant would have favorable effects on lipid and glucose metabolism; however, this variant actually seems to be associated with an unfavorable phenotype. Although Val162 is a gain-of-function variant with activating ligands, its basal transcription level is lower than that of the wild type. This might explain the differences between reports based on patient background. The results of the Framingham Heart Study indicated that Val162 is associated with greater TG and greater apolipoprotein CIII on low intake of polyunsaturated fatty acids (PUFA), a natural PPAR\(_\alpha\) ligand; in contrast, Val162 was associated with lower apolipoprotein CIII when PUFA intake was high\(^{48}\). Val162 was also shown to be associated with less progression of diffuse coronary atherosclerosis\(^{49}\).

**PPAR\(_\alpha\) Intron7 G>C**

The intron7 G>C variant (rs4253778) had also been investigated extensively. In subjects with type 2 diabetes, the G/G genotype was associated with a good response to fenofibrate treatment\(^{50}\). In cases of cardiac disease, intron7 C allele carriers showed greater coronary atherosclerosis progression\(^{49}\), premature coronary artery disease\(^{51}\) and left ventricular hypertrophy\(^{52}\). The mechanism by which this variant affects PPAR\(_\alpha\) function remains unclear.

**Other PPAR\(_\alpha\) Gene Variants**

The p.Gln395Glu variant was identified in our Japanese population study\(^{11}\). This variant was associated with higher LDL-C in patients with suspected coronary artery disease, and in vitro assay indicated it to be a gain-of-function variant\(^{10}\).

The p.Val227Ala variant has been reported in Asian populations\(^{53, 54}\). Ala227 was associated with lower total cholesterol (TC) and lower TG in Chinese women\(^{53}\), and with lower TC in a non-drinking group of Japanese men\(^{54}\). Ala227 was associated with a smaller waist circumference and non-alcoholic fatty liver disease in a Chinese population\(^{55}\). Ala227 showed reduced transactivation activity in an in vitro assay\(^{56}\). These data are consistent with higher TC or TG in individuals with PPAR\(_\alpha\) gain-of-function variants; however, the mechanism of the discrepancy, that higher activity PPAR\(_\alpha\) variants are associated with dyslipidemia, whereas PPAR\(_\alpha\) activating fibrates ameliorate dyslipidemia, remains to be elucidated.

**PPAR\(_\gamma\)**

Insulin-sensitizing thiazolidinediones (TZD) are ligands of PPAR\(_\gamma\). The PPAR\(_\gamma\)2 isoform is expressed solely in adipose tissues, and plays a critical role in adipogenesis. Some angiotensin-receptor-blockers, such as telmisartan, shows PPAR\(_\gamma\) activation, and are expected to have an additional advantage\(^{57}\). There is no doubt of the clinical usefulness of TZD in the amelioration of diabetes mellitus, but there is controversy regarding its efficacy in cardiovascular disease. Meta-analysis data have suggested possible adverse cardiovascular outcomes with rosiglitazone, in contrast with pioglitazone which shows protective effects in the secondary prevention of cardiovascular disease\(^{58-61}\). We must be aware that these meta-analyses were not conducted to compare rosiglitazone and pioglitazone, and the clinical settings may not have been comparable between these two drugs. Deeg et al. reported that at least their effects on lipoproteins were significantly different between type 2 diabetes and dyslipidemic patients in a head-to-head randomized clinical study\(^{62}\). Pioglitazone showed more favorable changes in VLDL, LDL and HDL than rosiglitazone.

Rosioglitazone is a specific ligand solely for PPAR\(_\gamma\), whereas pioglitazone partially activates PPAR\(_\alpha\). Pioglitazone treatment causes a reduction in plasma TG concentrations, while rosiglitazone had the opposite effect; these observations may be associated with the partial agonistic action of pioglitazone on PPAR\(_\alpha\) receptors. Clinical advantages may be influenced by the pharmacological differences between these drugs\(^{56-61}\). It is interesting that balanced agonists of PPARs may be better than highly specific ligands for one isoform in some clinical trials.

PPAR\(_\gamma\) is also expressed in the collecting ducts of the kidney, and TZD-induced edema can be accounted for by renal salt absorption\(^{69}\). There is a report that the use of rosiglitazone was associated with an increased risk of congestive heart failure and mortality compared with pioglitazone in a large cohort of elderly individuals with diabetes\(^{65}\). PPAR\(_\gamma\) has been shown to be a key regulator in differentiation toward adipocytes and to osteoblasts and osteoclasts\(^{66, 67}\). PPAR\(_\gamma\) activation enhances adipocyte differentiation and inhibits osteoblast differentiation from mesenchymal stem cells, and osteoclast maturation from precursors. It has recently been suggested that TZD-induced skeletal fragility\(^{68}\) may be attributable to these regulation systems.

**PPAR\(_\gamma\)2 p.Pro12Ala**

The common p.Pro12Ala in the PPAR\(_\gamma\)2 gene is the most extensively investigated polymorphism in RXR heterodimers; however, the clinical impact of this polymorphism is complex. The Ala12 variant shows moderately reduced transcriptional activity of
PPAR\textsubscript{\gamma}2\textsuperscript{69}, and is shown to be associated with lower BMI, increased insulin sensitivity, and being consequently protective against diabetes\textsuperscript{70}. Indeed, it has been reported that the Ala12 allele was associated with a reduced incidence of cardiovascular disease\textsuperscript{71-73}; however, in some large-scale studies, the Ala12 allele was also shown to be associated with unfavorable phenotypes, such as increased waist circumference or increased BMI\textsuperscript{74-80}. The potential mechanism of how this polymorphism would exert its effect is its influence on adipose tissue development and adipose-derived cytokines. Some investigators have reported that the Ala12 allele was associated with decreased plasma adiponectin levels in the Japanese population\textsuperscript{81, 82}. These inconsistencies were suggested to be because the protective effect of the Ala12 allele may be lost once diabetes has developed\textsuperscript{70}.

A recent meta-analysis of 32,000 non-diabetic subjects in 57 studies showed no clear association between diabetic traits and this polymorphism, but in selected subgroups, such as Caucasians and obese subjects, associations were observed between the Ala12 allele and both greater BMI and increased insulin sensitivity\textsuperscript{83}. Using stable isotope techniques, a mixed meal study \textit{in vivo} showed that Ala12 carriers had lower plasma fatty acid levels, higher adipose and muscle blood flow, and greater insulin sensitivity than Pro12 homozygotes\textsuperscript{84}. BMI and insulin sensitivity may counteract each other in subjects with the Ala12 allele.

Very recently, a study on Pro12Ala knockin mice was reported\textsuperscript{85}. In this animal model, Ala/Ala mice on a chow diet are leaner and have improved insulin sensitivity and plasma lipid profiles, but high-fat feeding eliminated these beneficial effects, with changes in cofactor interaction and adiponectin signaling. These data suggest the key role of gene-environment interactions, and may explain some inconsistencies in the human phenotype.

As TZD drugs are known to potentially induce edema, PPAR\textsubscript{\gamma} is expressed in the kidney. This can affect the function and prognosis of diabetic nephropathy, blood pressure, and also the prognosis of cardiovascular disease through chronic kidney disease. Recent studies showed that the Ala12 allele is associated with enhanced decline in the glomerular filtration rate and predicts end-stage renal disease, and is also associated with cardiovascular disease and mortality in diabetic patients with nephropathy\textsuperscript{86-88}; however, the opposite effect of this genotype on cardiovascular disease has been reported in subjects without chronic kidney disease\textsuperscript{71-73}. We speculate that the presence of nephropathic disease may affect the results of these controversial reports on this polymorphism.

With regard to the response to TZD treatment, subjects with the Ala12 allele showed a better response to therapy than those with Pro12 homozygotes in one study\textsuperscript{89}, and no difference was observed between the two genotypes in another\textsuperscript{90}; however, the Ala12 allele was the most important risk factor for TZD-induced edema\textsuperscript{91}. As PPAR\textsubscript{\gamma} plays an important role in osteocyte differentiation\textsuperscript{66, 67}, it is possible to speculate on its association with bone metabolism. Rhee \textit{et al.} reported that the Ala12 allele was associated with the serum osteoprotegerin level, a key inhibitor of osteoclastogenesis, in a Korean population\textsuperscript{92}.

Other PPAR\textsubscript{\gamma} Gene Variants

A gain-of-function p.Pro115Gln variant is associated with obesity and insulin resistance\textsuperscript{93, 94}. A silent c.161C>T (1431C>T: rs3856806) polymorphism in exon 6 of the PPAR\textsubscript{\gamma} gene was investigated in several studies. Doney \textit{et al.} reported that the T allele of this polymorphism is associated with increased body weight, but this allele is in strong linkage disequilibrium with the Pro12Ala variant, so they suggested that the opposing interaction between c.161C>T and Pro12Ala variant might be one reason for the inconsistent effect of these genotypes\textsuperscript{95}. They reported that the Ala12 allele was protective against diabetes in their study; however, this protective effect, attributable to the Ala12 variant, was absent when the Ala12 variant was present with the T allele of c.161C>T\textsuperscript{96}. However, no such opposite interaction or additive effects between c.161C>T and p.Pro12Ala was confirmed in another study, and the T allele was associated with a decreased risk of diabetes\textsuperscript{97}. Reduced coronary artery disease risk and lower TG were associated with the T allele\textsuperscript{98, 99}, but a cohort study also indicated an association with higher coronary risk\textsuperscript{100}. Another study suggested that, in obese subjects, the T allele was shown to be associated with higher plasma leptin levels\textsuperscript{100}. A series of rare loss-of-function mutations (p.Cys114Arg, p.Cys131Tyr, p.Cys162Trp, p.Cys190Ser, p.Val290Met, p.Ser315X, p.Arg357X, p.Phe388Leu, p.Arg425Cys, p.Pro467Leu) were reported in partial lipodystrophy patients, and most were described as dominant-negative type\textsuperscript{101, 102}. These patients showed insulin resistance and dyslipidemia. Lipodystrophic patients with p.Pro467Leu mutation showed high systemic non-esterified fatty acid (NEFA) because of the excessive and uncontrolled generation of NEFA directly from TG-rich lipoproteins\textsuperscript{103}.

PPAR\textsubscript{\delta}

PPAR\textsubscript{\delta} is ubiquitously expressed as a key regula-
tor of fatty acid oxidation and energy uncoupling. This less tissue-specific PPARδ has not been the focus of as much attention as PPARα and PPARγ as a therapeutic target; however, recent progress has shown that PPARδ critically regulates fatty acid oxidation and energy dissipation in skeletal muscle and adipose tissue, and should be a promising target in the treatment of metabolic syndrome. PPARδ modulates insulin sensitivity through the regulation of adipose tissue-and hepatic-resident macrophage regulation. Recently, PPARδ agonists have been reported to be “exercise mimetics” and increase exercise endurance without exercise in mice, suggesting a unique therapeutic approach for life-style-related disease. PPARδ also regulates multiple pro-inflammatory pathways to suppress atherosclerosis, and plays some roles in angiogenesis with endothelial progenitor cells. Recent investigations have revealed that PPARδ is intimately involved in metabolic regulation and atherosclerosis.

**PPARδ c.-87T>C**

No mutations resulting in amino acid substitution have yet been identified in the PPARδ gene, which was also the same in our study. Several silent nucleotide substitution and non-coding region polymorphisms in the PPARδ gene have been investigated for their clinical phenotype, but there have been few detailed studies regarding the functional consequences of these variants. Skogsberg et al. reported that in 4 polymorphisms, only the rare C allele of c.-87T>C in exon4 (also named +294T>C) showed a significant association with increased LDL-C levels in healthy men. The C allele was associated with higher transcriptional activity. This C allele of c.-87T>C polymorphism was also shown to be associated with unfavorable phenotypes, such as low HDL-C in women, and increased fasting plasma glucose.

On the other hand, from the analysis of 15 PPARδ polymorphisms among subjects without type 2 diabetes or hereditary dyslipidemia in French-Canadians, only the C allele of c.-87T>C showed an association with favorable phenotypes, such as a lower risk of metabolic syndrome, and lower BMI, the latter of which was reported in another Caucasian group.

From the standpoint of individual differences in responsiveness to 20 weeks of endurance exercise training, the C allele of -87T>C was associated not only with smaller training-induced increase in maximal oxygen consumption and lower training response in maximal power output, but also with a greater increase in plasma HDL-C with physical training (HERITAGE Family Study).

**Other PPARδ Gene Polymorphisms**

Several haplotype analyses with SNPs showed significant metabolic phenotypes even though functional consequences were not determined. Shin et al. reported that a major haplotype (ht1) with 5 SNPs was associated with lower BMI in Korean subjects. Vanttinen et al. reported that most common haplotype (named hgt1) with 3 SNPs was associated with lower skeletal muscle glucose uptake detected by FDG-PET scanning, higher systolic blood pressure, higher TC, and higher LDL-C in a Finnish population.

With regard to lifestyle intervention, Thamer et al. reported that carriers of minor allele 3 SNPs showed reduced responses in adipose tissue mass, hepatic lipids evaluated by MRI, and a reduced increase in muscle volume of the leg after 9-month exercise and a dietary lifestyle-intervention program.

As the PPARδ gene is expressed at high levels in skeletal muscle and adipose tissue, genetic studies suggested a significant genetic contribution of PPARδ to metabolic homeostasis, presumably modified by its interactions with lifestyle.

**LXRs**

LXRs and FXR have been described as cooperation regulators in cholesterol absorption, secretion, and synthesis. LXR is an oxysterol sensor, and its subfamily consists of LXRα and LXRβ. LXRα is expressed mainly in the liver, small intestine, macrophages and adipose tissue, and is the target gene of PPARγ, whereas LXRβ is expressed ubiquitously. In response to increased cellular oxysterols, LXRs activate genes involved in the "reverse cholesterol transport" system, cholesterol efflux from peripheral tissues or macrophages, transport in serum, uptake into liver, cholesterol catabolism into bile acids, biliary secretion of cholesterol, and inhibition of cholesterol absorption. LXRs also activate SREBP-1c, the master regulator of fatty acid synthesis. Studies in LXRα- and LXRβ-deficient mice suggest a more prominent role of LXRα than LXRβ in lipid metabolism, LXR agonists not only decrease cholesterol absorption and increase HDL-C with NPC1L1, but also induce hypertriglyceridemia, mostly via SREBP-1c activation, and this may potentially hamper drug development. Moreover, both LXR isoforms are expressed in skeletal muscle and are associated with lipid accumulation. Mitro et al. reported in Nature that LXR is also a glucose sensor, and can integrate hepatic glucose metabolism and fatty acid synthesis, but a contradictory paper by Denef et al. has also been published.
LXRs also play important roles in immune and inflammatory responses. We demonstrated their roles in innate immunity that LXR activation regulates TLR4 and potentiates immune responses in human macrophages.

**LXRA Gene Polymorphisms**

Recently, LXRA gene polymorphisms were reported to be associated with metabolic syndrome in two large populations. They genotyped 3 SNPs, and found that only the A allele of c.-6G>A polymorphism was associated with a 30% reduction in the risk of metabolic syndrome and an increase in plasma HDL-C. Human primary macrophages were analyzed for their mRNA levels of LXRA and ABCA1 as a target of LXRA, but no significant differences were confirmed. The c.-6G>A polymorphism is included in the Kozak sequence, which may affect translational efficiency.

LXRA c.-115G>A, c.-840C>A and c.-1830 T>C genotypes were analyzed in a French-Canadian population, and -155A, -180A and -1830C alleles were associated with higher plasma TC. High cholesterol intake was associated with higher TC in -155A carriers, suggesting that cholesterol intake interacts with LXRA variants to modulate lipid profiles. The LXRA rs2279238 SNP was shown to be associated with female obesity. The functional consequences of these SNPs were not determined.

Moreover, LXRA gene polymorphisms were shown to be associated with human life span in a population-based prospective follow-up study. Mooijaart et al. tagged four common haplotypes with 4 genotyp SNPs, and found that “haplotype 2” was associated with reduced mortality during 7-year follow-up, predominantly caused by reduced mortality from infectious disease. Haplotype 2 was also associated with higher levels of plasma apolipoprotein E, a target gene of LXRA, and higher levels of TG.

**LXRB Gene Polymorphisms**

A limited number of clinical studies indicated associations between LXRB and metabolic disorders. Eleven SNPs in LXRB were genotyped in obese and non-obese women, and 2 SNPs (LB44732G/A and rs2695121) were associated with obesity phenotypes. The functional consequences of these SNPs were not determined.

**FXR**

FXR is a bile acid sensor, which controls cholesterol and bile acid homeostasis through the entero-hepatic circulation in cooperation with LXRs, and also has important roles in glucose homeostasis.

Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. Chenodeoxycholic acid (CDCA) is the most potent natural agonist of human FXR and was evaluated to treat hypertriglyceridemia, but the TG-lowering effect was transient and showed side effects, such as liver toxicity or diarrhea.

Watanabe et al. reported that bile acids induce energy expenditure, preventing obesity and insulin resistance. Recently, bile acid-binding resins have attracted attention as a new means of preventing and treating obesity, insulin resistance, and diabetes. Bile acids are now appreciated as “complex metabolic integrators” and not just as lipid solubilizers or simple regulators of bile acid homeostasis, and bile acid signaling pathways have become attractive therapeutic targets of metabolic disorders, including obesity, type 2 diabetes, hypertriglyceridemia, atherosclerosis and non-alcoholic steatohepatitis, even though FXR may not be the main target of treatment.

**FXR Gene Polymorphisms**

There have been only a few reports regarding FXR gene polymorphisms and metabolic disorders. We identified a common c.-1G>T polymorphism of one nucleotide preceding the start codon in a Japanese population, and also in other ethnic groups. The T allele of the c.-1G>T polymorphism showed reduced transactivation, and was associated with decreased hepatic target gene expression. Further investigation regarding this polymorphism is on going.

**Conclusions**

The combined efforts of basic and clinical science regarding nuclear receptors have achieved significant progress in unraveling the intricately linked control system of energy expenditure, lipid and glucose homeostasis, inflammation, and atherosclerosis. It is quite interesting to see clinical studies of genetic variants of RXR heterodimers sometimes revealing results somehow contrary to those expected from the pharmacological effects of their activators. Understanding the cross-talk mechanisms with RXR heterodimers will provide new clues to uncover the background of multifactorial disease, such as metabolic syndrome or FCHL. There are still numerous unanswered questions, but the discovery of new pathways will lead to the establishment of new strategies for the prevention and treatment of dyslipidemia, obesity, diabetes, and cardiovascular disease.
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