Editorial Guest

Wnt signaling, a novel pathway for coronary artery disease and metabolic syndrome

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Genetic influences on coronary artery disease

Much of the reduction in the incidence of myocardial infarction is attributable to the identification of reversible coronary artery disease risk factors that have been identified by epidemiological studies. Genetic studies of rare families segregating single genes that impart very large effects on CAD risk factors have identified many genes and physiologic pathways[1-3]. For example, mutations that impair function of the LDL receptor or the LDL endocytosis machinery lead to hypercholesterolemia, mutations in ABCA1 impair the systemic cholesterol efflux pathway leads to low HDL and Tangier disease and mutations in *ABCG5/8* impair cholesterol efflux from the enterocyte and biliary system that leads to a disease called sitostrolemia. Finally, mutations in genes that impair glucose sensing or insulin secretion by pancreatic beta cells constitute Mendelian forms of diabetes mellitus[1-3]. These mutations account for only small proportion of all CAD cases. Surprisingly, many of these risk factors cluster with one another more often than expected by chance[4,5] ; while this clustering, referred to as the metabolic syndrome, is recognized to be a common cause of CAD, the molecular mechanisms that unify their association have been obscure. Following we will describe our identification of a gene responsible for CAD and metabolic syndrome discovered in an Iranian kindred. The mutation in this gene impairs a pathway known as Wnt signaling pathway. With the expansion of research in Wnt signaling pathways, our knowledge about the role of this pathway in physiological processes of glucose and lipid metabolism and the pathogenesis of diabetes and coronary artery disease continues to increase.

Identification of LRP6 mutation as a cause of coronary artery disease and metabolic syndrome

We have characterized a large Iranian family with autosomal dominant early CAD, features of the metabolic syndrome (hyperlipidemia, hypertension, and diabetes) and osteoporosis[6]. These traits showed genetic linkage to a short segment of chromosome 12p in which we identified a missense mutation in LRP6, which encodes a co-receptor in the Wnt signaling pathway (fig. 1). The mutation, which substitutes cysteine for arginine at a evolutionarily highly conserved residue of an epidermal growth factor-like domain, impairs the Wnt signaling in vitro.

Genotype phenotype correlation showed that the mutation impacts not only CAD but a constellation of risk factors (hypertension,

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Table 1. Comparison of phenotypes in carriers and non-carriers of LRP_{R611C}

Trait	LRP_{R611C} carriers	Non-carriers	P-value
LDL, mg/dl	170 ± 12	98 ± 5	$6x10^{-6}$
Triglycerides, mg/dl	209 ± 71	68 ± 20	$1x10^{-5}$
HDL, mg/dl	57 ± 8	56 ± 7	0.4
BMI, kg/m2	24.3 ± 2.6	24.4 ± 1.6	0.13
Systolic BP, mmHg	168 ± 21	116 ± 5	$8x10^{-5}$
Diastolic BP, mmHg	100 ± 14	81 ± 7	0.0025

hyperlipidemia, diabetes) that together are generally referred to as metabolic syndrome (Table 1). Our findings establish a causal link between impaired LRP6 function and early CAD and identify the Wnt signaling as a novel pathway involved in coronary artery disease and development of metabolic syndrome. Following we will describe different elements of Wnt signaling pathway and their relationship with the LRP6.

LRP6 and the WNT signaling pathway

Low density lipoprotein receptor (LDLR)-related protein LRP6 is a members of the expanding LDL receptor family[7-11]. LRP6 and LRP5 are indispensable co-receptors of the canonical Wnt pathway by interacting with several key components of the Wnt/ß-catenin signaling pathway (Fig. 2).

Wnts are secreted family of cysteine

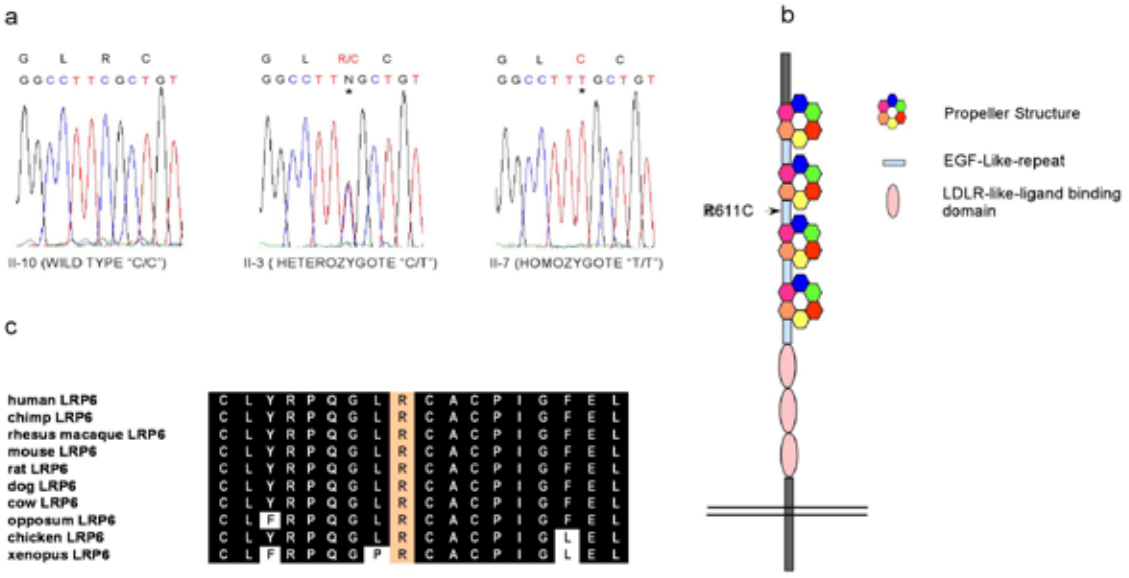

Figure 1: (a) The DNA sequence of a segment of LRP6 exon 9 in an unaffected (left) a heterozygous mutation carrier (middle) and the homozygous index case (right). (b) A schematic diagram of the structure of LRP6. (c) A portion of the second EGF-like domain of LRP6 in diverse vertebrate species.

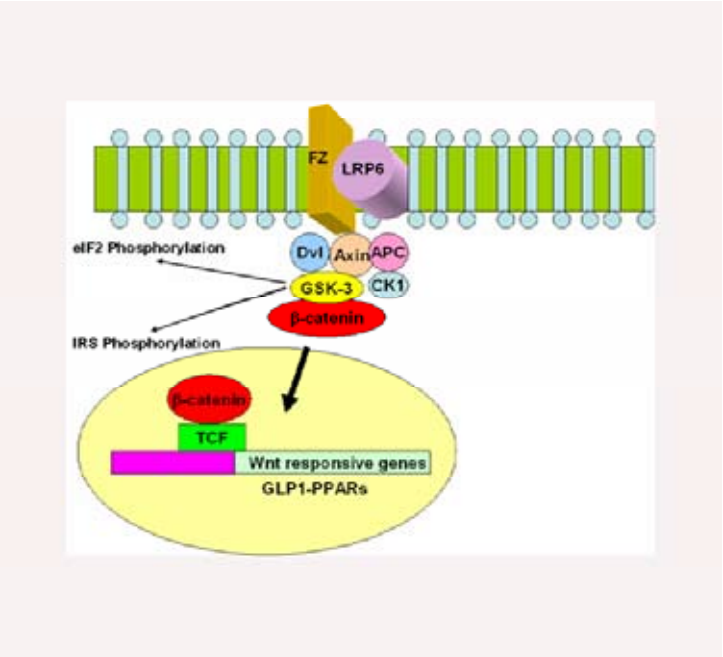

Figure 2: Wnt signaling pathway

rich signaling molecules associated with extracellular matrix that regulate diverse processes. Extensive work has been done in relation to the Wnt signaling and cell proliferation, migration, polarity, differentiation, axon outgrowth and regulation of bone metabolism[12-17]. Our findings in human in conjunction with recent works in mouse[18, 19] underscore the Wnt signaling's emerging involvement in diverse pathways such as glucose and lipid metabolism and atherosclerosis.

Wnts bind to two co-receptors, the Frizzled-type seven-transmembrane-domain receptor and the LRP 5/6[20](Fig.2). The canonical Wnt signaling pathway is characterized by a Wnt-dependent inhibition of glycogen synthase kinase-3ß (GSK-3ß), a serine theronine kinase which is assembled in a large cytoplasmic complex that includes Dishevelled, casein kinase I, Axin, APC, and CK1 [7-11]. These interactions cause ß-catenin stabilization by inhibiting its phosphorylation. As a consequence, cytoplasmic ß-catenin is translocated to the nucleus and forms a heterodimer complex with a family of high-mobility group-like transcription factors, including leukocyte enhancer factor-1 (LEF-1) and T-cell factors (TCF1-4)[21], activating transcription of target genes. LRP6 also binds to a number of naturally occurring antagonists of the Wnt signaling pathway that include Dickkopf (Dkk1 and Dkk2)[22]. Following we will describe our current knowledge about the role of Wnt signaling in different disease pathogenesis.

The Wnt signaling in diabetes

One of the target genes of TCF is proglucagon (*glu*) ,which is expressed in pancreatic islet α-cells, intestinal endocrine L cells, and selected neural cells in the brain[23]. Post-translational processing leads to the cell/tissue-specific biosynthesis of three major peptide hormones, glucagon, glucagon-like peptide-1 (GLP-1), and GLP-2. Glucagon is synthesized in the pancreatic α-cells and is a

counter-regulatory hormone to insulin. GLP-1 is produced in the gut and brain (and possibly pancreas) and lowers blood sugar levels through stimulation of insulin secretion in the postprandial state[24]and its biosynthesis by promoting pancreatic endocrine cell growth[25], and through tissue specific inhibition of glucagon release, increasing insulin sensitivity, and induction of satiety[26]. In glucose-intolerant Wistar rats GLP-1 activates insulin, *GLUT-2*, and glucokinase genes at the transcriptional level. This is associated with an expansion of beta-cell mass via islet-cell neogenesis [27, 28]. Similar findings are seen using the GLP-1 receptor agonist exendin-4 in partially pancreatectomized diabetic rats [28]. A GLP-1-dependent differentiation of pancreatic precursor cells into mature beta cells has also been proposed[29]. Finally, an inhibitory effect of GLP-1 on islet-cell apoptosis has been observed both *in vivo* and *in vitro* [30, 31]. Interestingly recent studies in human have shown that variant of TCF7L2 confer risk of diabetes on human[32, 33]. Mutations in several other TCFs have been associated with type 1 diabetes.

GSK3 is a key mediator of the Wnt signaling pathway in Glucose metabolism

Among the peptides in the Wnt signaling pathway GSK3 is of particular importance (Fig. 2). GSK3 is a regulator of cell survival and its overexpression induces apoptosis[34], by both inhibiting survival-promoting transcription factors such as CREB [35], and facilitating pro-apoptotic transcription factors, such as p53[36], especially during hypoxia [37] and ER stress [38]. Apoptosis of beta cell in diabetes is thought to be caused by phosphorylation of the initiation factor eIF2B by GSK3 [39].

One of the mechanisms contributing to insulin resistance is increased activity of GSK3. GSK3 action is increased in diabetic rodents[40] and in skeletal muscle from patients with type 2 diabetes. GSK3 inhibits glycogen synthesis and glucose uptake, alters the expression of genes regulated by insulin, and inhibits the insulin-receptor-coupled protein (IRS-1) via its phosphorylation [41]. Inhibitors of GSK3 enhance responses to insulin, lower blood glucose levels and stimulate glucose transport and glycogen synthesis in skeletal muscle from insulin-resistant Zucker rats, and increase IRS-1 expression and glucose uptake in human skeletal muscle. PPARs, a target gene of β-cathenin, promotes glycolysis in the liver, leading to reduced hepatic glucose output and lowering of blood glucose levels [42]. Future studies will certainly shed light on the exact molecular mechanism responsible for metabolic syndrome and coronary artery disease caused by LRP6 mutation.

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