Polymorphism

by Shahrul Rahman

Submission date: 06-Nov-2018 05:22PM (UTC+0700)
Submission ID: 1033903586
File name: Overview_Polymorphism_Full_Text.doc (54.5K)
Word count: 2270
Character count: 13093

Overview Polymorphism Gen LOX-1 3'UTR188C/T in Medan, Indonesia

Shahrul Rahman; Harun Al-Rasyid
Department of Internal Medicine, Medical Faculty, University Muhammadiyah Sumatera Utara, Medan, Indonesia

Abstract

AIM: LOX-1 belongs to the subgroup of scavenger receptor class E. Until now, there are 7 polymorphisms that have been identified in the LOX-1 gene. One is 3'UTR188C/T. The serum LOX-1 levels were significantly higher in NAFLD patients than in healthy controls. The serum LOX-1 levels could differentiate between steatohepatitis patients and healthy controls

Methods: Thirty-five participants (8 males and 27 females, mean age 43 ± 4.6 years) were recruited in this study. DNA was extracted from the peripheral blood leukocytes by the salting-out method. The LOX-1 3′UTR188CT genotypes were determined by PCR–RFLP technique. After RsaI restriction, two fragments were obtained: 184 and 23 bp for the C allele and a single fragment of 207 bp for the T allele. Fragments were visualized using ethidium bromide

Results and Discussion: From 35 subjects examined LOX-1 gene polymorphisms that have gained 3′UTR188 C/T CC genotype as many as 15 subjects (43%), CT 17 subjects (49%), and TT 3 subjects (8%). From 35 subjects who had a C allele were 47 (67%) and T alleles were 23 (33%).

Conclusion: CT genotype were most genotypes found in this study. C allele had a greater frequency than T allele

Keywords: Polymorphism; Non alcoholic fatty liver disease; allele

INTRODUCTION

NAFLD is the most common chronic liver disease in the world, affecting 20–30% of the general adult population. It encompasses a histologic spectrum ranging from simple steatosis to steatosis plus necroinflammation (NASH), the latter having different severities of fibrosis. NASH is associated with an increased risk of developing cirrhosis/end-stage liver disease, diabetes, and CVD (1); the correlation of liver histology in NAFLD with the severity of early atherosclerosis and with the impairment in glucose metabolism suggests that the same molecular mechanisms may underlie liver injury, diabetes, and atherogenesis (1). The knowledge of such mechanisms would help in selecting NASH subjects at greater risk of cardiometabolic and liver-related complications for early specific interventions and tight monitoring

High circulating concentrations of oxidized LDLs are associated with an increased risk of metabolic disorders, including obesity, hyperglycemia, hypertriglyceridemia, and CVD (2), with the putative mechanism being an increased cellular uptake of oxLDL, which triggers intracellular ROS production, cellular apoptosis, and inflammation. oxLDLs can also activate hepatic Kupffer and stellate cells and trigger inflammation and fibrogenesis (3, 4). Consistently, increased oxLDL concentrations have been shown to correlate with histologic severity in liver tissue samples from NAFLD patients (5)

LOX-1 receptor belongs to the C-type lectin-like receptors family. It is a type II membrane glycoprotein composed by 273 residues and four domains: a short N-terminal cytoplasmic domain (CYTO, 34 aa), a single transmembrane domain (TM, 26 aa), an extracellular region consisting of a coiled-coil domain named NECK (82 aa), and a C-type lectin-like domain (CTLD, 131 aa) at the C-terminus (6-8). The CTLD domain forms a disulfide-linked heart-

shaped homodimer, which assembles in larger functional oligomers through non-covalent interactions (6,7,9). The crystal structure of the human LOX-1 receptor revealed the presence of arginine residues, forming a basic spine located on the CTLD and crossing the entire dimer, which are engaged in the ligand binding (6-7). Molecular dynamics (MD) simulations evidenced that mutations of the basic spine residues Trp150 and Lys167 markedly reduces LOX-1 binding activity (10-13). A hydrophobic tunnel runs through the center of the CTLD. The tunnel access is surrounded by a quasi-conical surface where hydrophilic and hydrophobic patches are scattered. Mutations of residues that obstruct the tunnel and the presence of phospholipids that can interact with residues in the tunnel, significantly prevent the binding ability of LOX-1 receptor (14,15). The NECK domain appears as a dimer consisting of two α-helices wound in a parallel coiled-coil structure and displays specific residues, which functionally modulate the flexibility of this region (16).

LOX-1 receptor recognises a wide range of negatively charged substances. In addition to ox-LDL, these ligands include activated platelets, apoptotic bodies, bacteria, advanced glycation end products (AGEs), heat shock proteins (HSP60 and HSP70) and C-reactive protein (CRP) (12). Beside endothelial cells, LOX-1 is expressed in several cell types including smooth muscle cells, fibroblasts and platelets (14). Under physiological conditions, it is expressed at low level and it plays a versatile role in innate immunity or infections and may be involved in the removal of cellular debris and aged/apoptotic cells from blood circulation. LOX-1 expression is induced by ligand binding via intracellular signaling in multiple pathological states, such as atherosclerosis, obesity, inflammation and in cellular transformation and carcinogenesis (8-10). Its activation triggers the oxidative stress response causing plaque vulnerability and potential rupture, which leads to acute atherothrombotic vascular occlusion and tissue infarction (6,8). The higher local concentration of ox-LDL, together with the higher expression of LOX-1 receptor, in atherosclerotic lesions, provides a molecular basis linking ox-LDL to endothelial cells and the resultant cellular activation, dysfunction, and injury. In animal models, deletion of *orl1* gene in Ldlr knockout mice results in much smaller atherosclerosis lesions, drastic reduction of inflammation in aortic wall and of the extent of ischemia/reperfusion injury (5-6). LOX-1 is also expressed on the surface of immune cells such as denditric cells and macrophages where it contributes to inflammatory responses and foam cells formation, respectively. Ox-LDL binding to LOX-1 receptor induces the activation of membrane-bound NADPH oxidase leading to a rapid increase of reactive oxygen species (ROS) and the consequent induction of the redox-sensitive NF-kB pathway. Among the effects of the activation of NF-kB is the enhancement of pro-inflammatory cytokine and chemokine expression, such as MCP-1, VCAM-1, ICAM-1, E-selectin and P-selectin, with an increase of monocytes recruitment to endothelial cells (7-10).

METHODS

Thirty-five participants (8 males and 27 females, mean age 43 ± 4.6 years) were recruited in this study. DNA was extracted from the peripheral blood leukocytes by the salting-out method. The LOX-1 3′UTR188CT genotypes were determined by PCR–RFLP technique. After RsaI restriction, two fragments were obtained: 184 and 23 bp for the C allele and a single fragment of 207 bp for the T allele. Fragments were visualized using ethidium bromide.

The exclusion criteria were as follows: obesity [BMI (in kg/m^2) ≥30], diabetes (fasting plasma glucose ≥126 mg/dL or plasma glucose ≥200 mg/dL at >2 h on OGTT or antidiabetic drugs) or overt dyslipidemia (fasting serum cholesterol ≥200 mg/dL or plasma triglyceride ≥200 mg/dL); exposure to occupational hepatotoxins or drugs known to be steatogenic, to be hepatotoxic, or to affect lipid or glucose metabolism; positive autoimmune or celiac disease markers; or abnormal copper metabolism, serum α_1-antitripsin, or thyroid function.

Data were expressed as means ± SEMs. Differences across groups were analyzed by ANOVA and then by followed by Bonferroni correction, when variables were normally distributed; otherwise the Kruskal-Wallis test, followed by the post hoc Dunn test, was used to compare nonparametric variables. Normality was evaluated by using the Shapiro-Wilk test. Fisher's or chi-square test were used to compare categorical variables, as appropriate

RESULTS

From 35 subjects examined LOX-1 gene polymorphisms that have gained 3′UTR188 C/T CC genotype as many as 15 subjects (43%), CT 17 subjects (49%), and TT 3 subjects (8%). From 35 subjects who had a C allele were 47 (67%) and T alleles were 23 (33%).

Patients with NASH had higher plasma nitrotyrosine and insulin and lower HDL cholesterol and adiponectin concentrations than did the control subjects. Systolic and diastolic blood pressures were no different in the NASH patients than in the control subjects, and they were higher in LOX-1 IVS4-14 *G*-allele carriers than in homozygous *AA* carriers within the patient and control groups. On the basis of Adult Treatment Panel III criteria for the definition of the metabolic syndrome, 28% of NASH patients and 5% of control subjects had the whole spectrum of the metabolic syndrome

The presence of the LOX-1 allele was associated with the severity of liver steatosis, but not difference significantly in necroinflammation, and fibrosis in NASH patients, whereas the LIC and HII were comparable across LOX-1 genotypes (data not shown).

DISCUSSION

The novel findings of our study are the following: CT genotype were most genotypes found in this study. C allele had a greater frequency than T allele. LOX-1 may also promote liver disease indirectly, through modulation of pro- and antiinflammatory adipokines, which play a key role in the pathogenesis of liver injury in NASH (15–16). In particular, an enhanced LOX-1–mediated adipocyte uptake of circulating oxLDLs may impair the secretion of the antioxidant, antiinflammatory adipokine adiponectin and enhance secretion of proinflammatory cytokines, such as resistin (3–5). Remarkably, the analysis of postprandial adiponectin, resistin, and cytokeratin-18 fragment responses supports a crucial role for LOX-1 in mediating a high-fat diet–induced inflammation and liver injury (4, 13). Despite comparable fasting adipokine concentrations, the presence of the *G* allele induced a proinflammatory cytokine pattern postprandially, consisting of lower adiponectin and higher resistin concentrations.

The effect of LOX-1 IVS4-14 *A*→*G* SNP on lipoprotein subfraction metabolism after fat ingestion in humans is also unknown. In our subjects, the *G* allele was associated with postprandial small TRLP accumulation. Because small TRLPs are represented by the atherogenic remnants, this may be a novel mechanism connecting LOX-1 polymorphism to CVD risk. Several potential mechanisms may link LOX-1 polymorphism to small TRLP accumulation: it was previously shown that remnant particles are ligands for LOX-1 receptor and can thereby induce the production of ROS and proinflammatory cytokines by endothelial cells and migration of vascular smooth muscle cells (35, 36). It can therefore be speculated that the higher affinity for oxLDLs of LOX-1 receptor form encoded by the *G* allele may lead to preferential uptake of oxLDLs and delay clearance of TRLP remnants or, alternatively, that the increased uptake of oxLDLs by the liver may enhance hepatic secretion of small VLDL particles, which compete with chylomicron remnants for their clearance postprandially. This results in small TRLP accumulation. Clearly, further in vitro and in vivo kinetic studies need to clarify the functional pathways connecting LOX-1 *A*→*G*polymorphism to TRLP lipoprotein metabolism.

REFERENCES

1. Musso G, Gambino R, Cassader M, Pagano GF. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity, *Ann Med*

2. Holvoet P, De Keyzer D, Jacobs DR. Oxidized LDL and the metabolic syndrome. *Future Lipidol* 2008; 3 637–49

3. Lu L, Zeng M Li J, Hua J, Fan J, Fan Z, Dai N, Qiu D, Xiao S. Effects of Kupffer cells stimulated by triglyceride and very low-density lipoprotein on proliferation of rat hepatic stellate cells. *Chin Med J (Engl)* 1999; 112: 325–9

4. Kang Q, Chen A. Curcumin eliminates oxidized LDL roles in activating hepatic stellate cells by suppressing gene expression of lectin-like oxidized LDL receptor-1. *Lab Invest* 2009;89:1275–90

5. Ustundag Y, Ozer IO, Yenidunia S. Oxidized low density lipoprotein in liver tissue samples of our NASH patients, alcoholic and chronic viral hepatitis cases. *Gastroenterology* 2002;57:203

6. I. Ohki, T. Ishigaki, T. Oyama, S. Matsunaga, Q. Xie, M. Ohnishi-Kameyama, T. Murata, D. Tsuchiya, S. Machida, K. Morikawa and S. Tate, Crystal structure of human lectin-like, oxidized low-density lipoprotein receptor 1 ligand binding domain and its ligand recognition mode to OxLDL, Structure 13 (2005), 905–917. doi:10.1016/j.str.2005.03.016

7. H. Park, F.G. Adsit and J.C. Boyington, The 1.4 angstrom crystal structure of the human oxidized low density lipoprotein receptor LOX-1, J Biol Chem 280 (2005), 13593–13599. doi:10.1074/jbc.M500768200.

8. T. Sawamura, N. Kume, T. Aoyama, H. Moriwaki, H. Hoshikawa, Y. Aiba, T. Tanaka, S. Miwa, Y. Katsura, T. Kita and T. Masaki, An endothelial receptor for oxidized low density lipoprotein, Nature 386 (1997), 73–77. doi:10.1038/386073a0.

9. S. Biocca, I. Filesi, R. Mango, L. Maggiore, F. Baldini, L. Vecchione, A. Viola, G. Citro, G. Federici, F. Romeo and G. Novelli, The splice variant LOXIN inhibits LOX-1 receptor function through hetero-oligomerization, Journal of Molecular and Cellular Cardiology 44 (2008), 561–570. doi:10.1016/j.yjmcc.2007.11.017.

10. S. Biocca, M. Falconi, I. Filesi, F. Baldini, L. Vecchione, R. Mango, F. Romeo, G. Federici, A. Desideri and G. Novelli, Functional analysis and molecular dynamics simulation of LOX-1 K167N polymorphism reveal alteration of receptor activity, PLoS One 4 (2009), e4648. doi:10.1371/journal.pone.0004648.

11. M. Falconi, S. Biocca, G. Novelli and A. Desideri, Molecular dynamics simulation of human LOX-1 provides an explanation for the lack of OxLDL binding to the Trp150Ala mutant, *BMC Struct Biol* 7 (2007). doi:10.1186/1472-6807-7-73.

12. I. Ohki, H. Amida, R. Yamada, M. Sugihara, T. Ishigaki and S. Tate, Surface plasmon resonance study on functional significance of clustered organization of lectin-like oxidized LDL receptor (LOX-1), Biochim Biophys Acta 1814 (2011), 345–354. doi:10.1016/j.bbapap.2010.10.006.

13. I. Ohki, T. Ishigaki, T. Oyama, S. Matsunaga, Q. Xie, M. Ohnishi-Kameyama, T. Murata, D. Tsuchiya, S. Machida, K. Morikawa and S. Tate, Crystal structure of human lectin-like, oxidized low-density lipoprotein receptor 1 ligand binding domain and its ligand recognition mode to OxLDL, Structure 13 (2005), 905–917. doi:10.1016/j.str.2005.03.016.

14. M. Falconi, S. Ciccone, P. D'Arrigo, F. Viani, R. Sorge, G. Novelli, P. Patrizi, A. Desideri and S. Biocca, Design of a novel LOX-1 receptor antagonist mimicking the natural substrate, Biochem Biophys Res Commun 438 (2013), 340–345. doi:10.1016/j.bbrc.2013.07.073

15. O.L. Francone, M. Tu, L.J. Royer, J. Zhu, K. Stevens, J.J. Oleynek, Z. Lin, L. Shelley, T. Sand, Y. Luo and C.D. Kane, The hydrophobic tunnel present in LOX-1 is essential for oxidized LDL recognition and binding, J Lipid Res 50 (2009), 546–555. doi:10.1194/jlr.M800474-JLR200.

16. T. Ishigaki, I. Ohki, N. Utsunomiya-Tate and S. Tate, Chimeric structural stabilities in the coiled–coil structure of the NECK domain in human lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1), J Biochem 14 (2007), 855–866. doi:10.1093/jb/mvm093.

..

Polymorphism

ORIGINALITY REPORT

27%	17%	21%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Silvia Biocca, Tania Arcangeli, Elisa Tagliaferri, Barbara Testa et al. "Simulative and experimental investigation on the cleavage site that generates the soluble human LOX-1", Archives of Biochemistry and Biophysics, 2013 Publication	2%
2	f1000.com Internet Source	2%
3	Giovanni Musso. "Noninvasive assessment of liver disease severity with liver fat score and CK-18 in NAFLD: Prognostic value of liver fat equation goes beyond hepatic fat estimation", Hepatology, 02/2010 Publication	1%
4	Musso, Giovanni. "The Finnish Diabetes Risk Score (FINDRISC) and other non-invasive scores for screening of hepatic steatosis and associated cardiometabolic risk", Annals of Medicine, 2011. Publication	1%

5	Genc, Halil, Teoman Dogru, Gurkan Celebi, Serkan Tapan, Muammer Ercin, Fatih Ors, Cengizhan Acikel, Yusuf Demirci, Hasan Gurel, and Sait Bagci. "Non-alcoholic fatty liver disease per se is not associated with carotid atherosclerosis", Gulhane Medical Journal, 2013. Publication	1%
6	www.jove.com Internet Source	1%
7	apdw2016.org Internet Source	1%
8	Sofia Raniolo, Giulia Vindigni, Valeria Unida, Alessio Ottaviani, Elena Romano, Alessandro Desideri, Silvia Biocca. "Entry, fate and degradation of DNA nanocages in mammalian cells: a matter of receptors", Nanoscale, 2018 Publication	1%
9	Submitted to Higher Education Commission Pakistan Student Paper	1%
10	Submitted to University of Witwatersrand Student Paper	1%
11	www.scribd.com Internet Source	1%

12	Federico Iacovelli, Fabio Giovanni Tucci, Gabriele Macari, Mattia Falconi. "Multiple molecular dynamics simulations of human LOX-1 and Trp150Ala mutant reveal the structural determinants causing the full deactivation of the receptor", Proteins: Structure, Function, and Bioinformatics, 2017 Publication	1%
13	academic.oup.com Internet Source	1%
14	dadun.unav.edu Internet Source	1%
15	Tomoko Ishigaki. "Purification, crystallization and preliminary X-ray analysis of the ligand-binding domain of human lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1)", Acta Crystallographica Section F Structural Biology and Crystallization Communications, 4/28/2005 Publication	1%
16	Fangming Li. "Free cholesterol-induced macrophage apoptosis is mediated by inositol-requiring enzyme 1 alpha-regulated activation of Jun N-terminal kinase", Acta Biochimica et Biophysica Sinica, 3/2008 Publication	1%

17	discovery.ucl.ac.uk Internet Source	1%
18	ajpheart.physiology.org Internet Source	1%
19	oatext.com Internet Source	1%
20	Submitted to 60556 Student Paper	1%
21	www.intechopen.com Internet Source	1%
22	jme.endocrinology-journals.org Internet Source	1%
23	J. H. Dominguez. "Anti-LOX-1 therapy in rats with diabetes and dyslipidemia: ablation of renal vascular and epithelial manifestations", AJP Renal Physiology, 11/14/2007 Publication	1%
24	Bushra Zeya, Albina Arjuman, Nimai Chand Chandra. "Lectin-like Oxidized Low-Density Lipoprotein (LDL) Receptor (LOX-1): A Chameleon Receptor for Oxidized LDL", Biochemistry, 2016 Publication	1%
25	preview-bmcstructbiol.biomedcentral.com Internet Source	1%

26	Biocca, S.. "The splice variant LOXIN inhibits LOX-1 receptor function through hetero-oligomerization", Journal of Molecular and Cellular Cardiology, 200803 Publication	1%
27	"Scientists at University of Turin Report Research in Gastroenterology.(Report)", Gastroenterology Week, Dec 31 2012 Issue Publication	1%
28	eu-acme.org Internet Source	<1%
29	arno.unimaas.nl Internet Source	<1%
30	Fu, Rong, Tianhua Yan, Qiujuan Wang, Qinglong Guo, Hequan Yao, Xiaoming Wu, and Yang Li. "Suppression of endothelial cell adhesion by XJP-1, a new phenolic compound derived from banana peel", Vascular Pharmacology, 2012. Publication	<1%
31	content.karger.com Internet Source	<1%
32	Lubrano, Valter, and Silvana Balzan. "LOX-1, a new marker of risk and prognosis in coronary artery disease?", Molecular and Cellular Biochemistry, 2013.	<1%

Publication

33	H. H. Shih. "CRP is a novel ligand for the oxidized LDL receptor LOX-1", AJP Heart and Circulatory Physiology, 05/01/2009 Publication	<1%
34	Matarazzo, S., M. C. Quitadamo, R. Mango, S. Ciccone, G. Novelli, and S. Biocca. "Cholesterol-Lowering Drugs Inhibit Lectin-Like Oxidized Low-Density Lipoprotein-1 Receptor Function by Membrane Raft Disruption", Molecular Pharmacology, 2012. Publication	<1%

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		