

Bioinformatics Analysis of LDLR Gene Mutation That Shed A New Light On Red Yeast Rice Monacolin K Treatment – Systematic Review

Abstract:

Background: Hypercholesteremia is the major cause of cardiovascular diseases. It results from elevated cholesterol levels in the blood. LDL cholesterol is removed from the circulation by using the LDL receptor. Red mold rice or red yeast rice is produced by fermentation of the *Monascus Purpureus* yeast on rice. Many researchers suggest that the active component in Red Yeast Rice (monacolin k) serves as a treatment for hypercholesteremic patients.

Method: By using NCBI databases, specifically GenBank to analyze DNA sequence and mRNA sequence of LDLR gene. GenBank file format was helpful to extract an accession number of the gene, number of amino acids, exons, and length of nucleotides. FASTA format was also useful to retrieve the nucleotide sequence and get the function of the protein. BLAST was used to compare the protein product of the LDLR gene between humans and *paniscus* (pygmy chimpanzee).

Results: In accession number NC_000019, the number of amino acids in protein product is 44389 bp, and the number of exons found is 18. On the other hand, the gene is located in chromosome 19. The function of LDLR gene is to control the production of LDL receptor where the low-density lipoprotein particles attach to it and are taken into the cell ending up in the lysosome where the protein is degraded and cholesterol is made which will inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase that controls the production of cholesterol. Finally, many organisms have the same gene like dogs, cows, mice, rats, zebrafish, and frogs.

Conclusion: Mutation in the LDLR gene causing high level of cholesterol in the blood especially LDL (Low-density Lipoprotein). Monacolin k that found in red yeast rice (RYR) is safe and natural alternative treatment for hypercholesteremic patients by lowering the cholesterol level in the blood.

Keywords: LDL (Low-density Lipoprotein), LDLR (Low-density Lipoprotein Receptor), Hypercholesteremia, RYR (Red Yeast Rice), Monacolin k.

INTRODUCTION:

Cholesterol is a type of lipid that mostly produced by the liver. It is so important for cell membrane structure and cell function, cell signaling, morphogenesis, intestinal lipid digestion and absorption, reproduction, and stress response. Also, it plays a main role in sodium and water balance as well as calcium and phosphorous metabolism (Cortes et al., 2014). There are good and bad cholesterol in the blood, LDL Cholesterol (low-density lipoprotein) is the bad cholesterol, and HDL Cholesterol (high-density lipoprotein) is the good one. However, if cholesterol levels increased more than normal, it can block the blood vessel, which leads to cardiovascular diseases (WOODS, 2002). LDL is a lipoprotein particles with about 19-22 nm in diameter that transport lipid in a micelle-like arrangement. The surface of LDL contains alpha-helices and beta-sheets that have an amphipathic nature (the ability to interact with both lipids in the hydrophobic core and water outside the lipoprotein particle). On the other hand, the main part of LDL is apoB-100 which is an apolipoprotein that maintains the structural integrity of LDL and serves as ligands for LDL receptors to remove LDL from the blood circulation (Wolska and Remaley, 2020). The low-density lipoprotein receptors located on the outer surface of many types of cells. LDLRs play an important role in cholesterol hemostasis. Any mutations in the LDLR gene will lead to lifelong elevation of low-density lipoprotein cholesterol levels, which can increase premature coronary artery disease. Moreover, this genetic disorder is known as Familial hypercholesterolemia (FH) (Moradi et al., 2021). Over 1000 different mutations in the LDLR gene on the distal short arm of chromosome 19 (p13.1 - p13.3) have been described to date. The second gene responsible for fewer than 10% of FH cases encodes the ligand for LDLR, namely Apolipoprotein B-100 (ApoB- 100), located on the short arm of chromosome 2 (p24). Mutations in this gene reduce ligand affinity for the receptors and cause reduced clearance of LDL particles resulting in hypercholesterolemia, although normal LDLR activity. A mutation in the codon for amino acid 3500 (CGG-to-CAG) was found to be a CG mutation hotspot associated with defective LDLs and hypercholesterolemia. The pathophysiological consequences of LDLR or ApoB mutations are loss of protein function, which leads to monogenic FH. Defects in a third gene, located on the short arm of chromosome 1 (p34.1- p32), have also been identified to cause monogenic FH (AL-Allaf et al., 2010).

Elevated cholesterol level is the major cause of cardiovascular diseases. Statins, is a class of lipid-lowering medications that reduce illness and mortality in those who are at high risk of cardiovascular disease. They are the most common cholesterol-lowering drugs.[1]

cholesterol-lowering drugs like simvastatin, pravastatin, and atorvastatin, are the first-choice treatments used to reduce the chance of developing cardiovascular diseases (Taylor et al., 2013). Statins reduce total cholesterol and LDL-C by inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (Karr, 2017). An intervention review study shows that Statins, cholesterol-lowering drugs, had many side effects like inducing the onset of type II diabetes and Hemorrhagic stroke because some patients were non-adherent or resistant which led to poor outcomes. (Taylor et al., 2013). Red mold rice or red yeast rice is produced by fermentation of the *Monascus Purpureus* yeast on rice. It is one of the traditional medicines in the traditional Chinese medication (Heber et al, 1999). It was used for the first time during the Tang Dynasty in 800 AD as a food preservative and to make rice wine with red color. Lately, it has been found that red yeast rice was proven to have an effect that reduces the cholesterol in the blood (Hoofnagle, 2013).

In this review, five articles were analyzed. We aim to find out the efficacy of Red yeast rice in hypercholesteremic patients. The objectives are to analyze five articles that discuss the effect of RYR on total cholesterol, total triglyceride, and Low-density lipoprotein cholesterol. The question from this study is; what is the effect of RYR on hypercholesteremic patients?

UNDER PEE

Method:

Bioinformatics is a field of science that use computational programs to collect, store, and analyze a large amount of medical and biological data. In this review study, we found that there is a correlation between LDLR gene mutation and statin-Rice yeast. NCBI database was used to analyze the DNA and mRNA sequences. In the Gene bank database, we found that the accession number is NC_000019, the number of amino acids in protein product is 44389 bp, and the number of exons found is 18. The length of nucleotide is 58617616pb and the gene is located in chromosome 19 (p 3.2.).

The nucleotide sequence in FASTA format

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>NC_000019.10:11089432-11133820 Homo sapiens chromosome 19, GRCh38.p13
Primary Assembly
ATTGAAATGCTGTAAATGACGTGGGCCCCGAGTGCAATCGCGGGAAGCCAGGGTTTCCAGCTAGGACACA
GCAGGTCGTGATCCGGGTCGGGACACTGCCTGGCAGAGGCTGCGAGCATGGGGCCCTGGGGCTGGAAATT
GCGCTGGACCGTCGCCTTGCTCCTCGCCGCGGCGGGGACTGCAGGTAAGGCTTGCTCCAGGCGCCAGAAT
AGGTTGAGAGGGAGCCCCGGGGGGCCCTTGGAATTTATTTTTTTGGGTACAAATAATCACTCCATCCC
TGGGAGACTTGTGGGGTAATGGCACGGGGTCCTTCCCAAACGGCTGGAGGGGGCGCTGGAGGGGGCGCT
GAGGGGAGCGCAGGGTTCGGGAGGAGTCTGAGGGATTTAAGGGAAAACGGGGCACCGCTGTCCCCCAAGTC
TCCACAGGGTGAGGGACCGCATCTTCTTTGAGACGGAGTCTAGCTCTGTGCGCCAGGATGGAGTGCAGTG
GCACGATCTCAGCTCACTGCAACCTCCGCTCCCGGGTTTAAGCGAGTCTCCTCTCTCAGCCTCCCGAAT
AGCTGGGATTACAGGCGCCCAACCACCACGCCCCGCTAATTTTTGTATTTTAGTAGAGACGGGTTTTCA
CCATTTTGGCCAGGCTGGTCTCGAACCCCGACCTCAGGTGATCTGCCCCAAAAGTGCTGGGATTACAGGCG
TCAGCCACCGCGCCCGGGACCTCTCTTCTAAGTTCGGAGCTGGGTGTGGGGACCTCCAGTCTCTAAA
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The nucleotide sequence in FASTA format for mRNA

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>NM_000527.5 Homo sapiens low-density lipoprotein receptor (LDLR),
transcript variant 1, mRNA
GTGCAATCGCGGGAAGCCAGGGTTTCCAGCTAGGACACAGCAGGTCGTGATCCGGGTCGGGACACTGCCT
GGCAGAGGCTGCGAGCATGGGGCCCTGGGGCTGGAAATTGCGCTGGACCGTCGCCTTGCTCCTCGCCGCG
GCGGGGACTGCAGTGGGCGACAGATGCGAAAGAAACGAGTTCCAGTGCCAAGACGGGAAATGCATCTCCT
ACAAGTGGGTCTGCGATGGCAGCGCTGAGTGCCAGGATGGCTCTGATGAGTCCCAGGAGACGTGCTTGTC
TGTCACCTGCAAATCCGGGGACTTCAGCTGTGGGGGCGCTGTCAACCGCTGCATTCCCTCAGTTCCTGGAGG
TGCGATGGCCAAGTGGACTGCGACAACGGCTCAGACGAGCAAGGCTGTCCCCCAAGACGTGCTCCCAGG
ACGAGTTTCGCTGCCACGATGGGAAGTGCATCTCTCGGCAGTTTCGTCTGTGACTCAGACCGGGACTGCTT
GGACGGCTCAGACGAGGCCTCCTGCCCCGGTGCTCACCTGTGGTCCCGCCAGCTTCCAGTGCAACAGCTCC
ACCTGCATCCCCAGCTGTGGGCCTGCGACAACGACCCCGACTGCGAAGATGGCTCGGATGAGTGGCCGC
AGCGCTGTAGGGGTCTTTACGTGTTCCAAGGGGACAGTAGCCCCCTGCTCGGCCTTCGAGTTCCACTGCCT
AAGTGGCGAGTGCATCCACTCCAGCTGGCGCTGTGATGGTGGCCCCGACTGCAAGGACAAATCTGACGAG
GAAAACTGCGCTGTGGCCACCTGTGCGCCCTGACGAATTCCAGTGCTCTGATGGAACTGCATCCATGGCA
GCCGGCAGTGTGACCGGGAATATGACTGCAAGGACATGAGCGATGAAGTTGGCTGCGTTAATGTGACACT
CTGCGAGGGACCCAACAAGTTCAAGTGTACAGCGGCGAATGCATCACCCCTGGACAAAGTCTGCAACATG
GCTAGAGACTGCCGGGACTGGTCAGATGAACCCATCAAAGAGTGCGGGACCAACGAATGCTTGGACAACA
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The LDLR gene is located on chromosome 19. It consists of cell surface protein in cell-mediated endocytosis of specific ligands. It controls the production of LDL receptor where the low-density lipoprotein particles attach to it and are taken into the cell ending up in the lysosome where the

protein is degraded and cholesterol is made which will inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase that controls the production of cholesterol. Mutations in this gene cause the autosomal dominant disorder, familial hypercholesterolemia. Many organisms have the same gene like dogs, cows, mice, rats, zebrafish, and frogs.

By using BLAST to retrieve the sequences in FASTA format and find the homologous LDLR gene in other organisms. It was found that pan paniscus (pygmy chimpanzee) has 100% identities and 0% gaps. There are homology and difference in protein products in LDLR genes in humans and other organisms.

3. Result and Discussion:

3.1 Mechanism of action:

Hypercholesterolemia results from high cholesterol levels in the blood. Cholesterol is produced by the conversion of acetyl CoA and Acetoacetyl-CoA to Mevalonate using HMG CoA Reductase. Monacolin K in Red rice yeast will inhibit HMG-CoA reductase. As result, it will prevent the conversion of 3-hydroxy-3-methylglutaryl CoA to mevalonate and reduce the production of cholesterol (Musselman, Pettit, and Derenski, 2011). See figure 1.

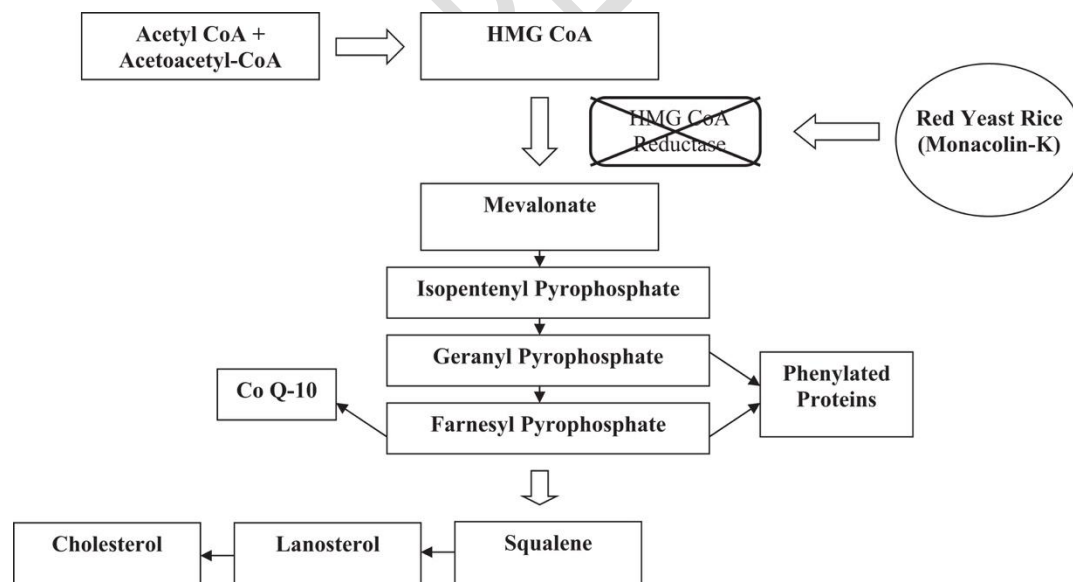


Figure 1, Red yeast rice effects on cholesterol biosynthesis according to (Musselman, Pettit, and Derenski, 2011, p.34.)

3.2 Clinical trials:

In Japan, randomized control trial was conducted that include eighteen patients suffering from mild dyslipidemia, LDL cholesterol between 3.62 mmol/L and 4.65 mmol/L, no history of cardiovascular diseases, and age between 20 and 80 years. Patients were randomized to receive low-dose red yeast rice (200mg/day) containing 2mg monacolin K or diet therapy alone for 8 weeks. Supplementation with red yeast rice significantly decreased the low-density lipoprotein compared to the dietary group ($p=0.002$ at 4 weeks\ $p=0.030$ at 8 weeks). It also shows a decrease in total cholesterol ($p<0.001$ at 4 weeks, $p=0.014$ at 8 weeks), triglycerides ($p=0.665$ at 4 weeks= 0.505 at 8 weeks), and High-density lipoprotein cholesterol ($P=0.038$ at week 4, $p=0.082$ at week 8), Apolipoprotein. There are reductions in systolic blood pressure ($p=0.040$) and diastolic blood pressure ($p=0.018$) were significantly greater in the red yeast rice group than in the control group after 8 weeks. It has no adverse effect on organ function. (Minamizuka et al., 2021)

Based on positive results in using nutraceutical compounds containing red yeast rice extracts providing a high daily dose of 2.5–10 mg of monacolin K. Sixty hypercholesteremic and hypertensive patients aged ≥ 18 years, with hypocholesterolemia evaluated as the total cholesterol serum level ≥ 200 mg dl⁻¹ and the LDLC serum level range between 130 and 190 mg dl⁻¹ were enrolled to the clinical trials for one month. 30 participants received 333 mg red yeast rice (10 mg monacolin K), 20 mg policosanols (12 mg octacaine), 20 mg resveratrol, 50 μ g chromium picolinate, and 3.15 mg black pepper (2.99 mg piperine) were added to the compound to improve the enteric level absorption of resveratrol. While the control group, composed of 30 participants followed-up only with a diet program. A significant reduction of TC, TG, and LDLC was noticed in both groups but the group that received monacolin showed a significantly greater reduction ($p < 0.001$) compared to the control group ($p < 0.05$). No difference was found for HDLC value in both groups. Moreover, the concentration of TG was reduced by 5.0% in the supplement group for the whole 12 weeks and no change was observed in TG in the placebo group. Also, it does not show any side effects. (Mazza et al., 2018)

In a randomized-placebo controlled intervention study done in Germany, one hundred forty-two participants that are not under statin therapy, with LDL-C level ≥ 4.14 mmol/L and ≤ 5.69 mmol/L and aged between 18 and 70 years were randomly selected to receive either 3 mg monacolin K and 200 μ g folic acid per day or placebo for 12 weeks. A remarkable reduction ($P < .001$) of LDL-C (-14.8%), and total cholesterol (-11.2%) in the RYR group after 12 weeks. Furthermore, 51% of

the participants of the RYR group achieve the limit of LDL-C < 4.14 mmol. However, no significant change was observed in the placebo group. No adverse effect of RYR was noticed during 12 weeks. (Heinz et al., 2016)

Twenty-five physicians joined in a double-blind placebo control trial. Thirty-one were instructed to take two capsules every evening for eight weeks (each capsule had 5,025 mg of Monacolin K). Twenty-one received placebo. At the end of week eight, the intervention group showed low LDL-C (22%) and total cholesterol (15%) compared to the control group which showed no changes ($p < 0.001$). According to systematic Coronary risk evaluation, five out of thirty-one participants in intervention show reduce risk of cardiovascular as a result of low cholesterol levels. (Verhoeven et al., 2013).

Another study performed in India included 191 patients with a mean baseline of LDL cholesterol of more than 120mg/dl. Patients were randomized to receive either 400 mg of red rice yeast twice daily or placebo for a total of 12 weeks. Furthermore, comparing to baseline, LDL cholesterol was reduced by 22% ($P < 0.0001$), non-HDL-C by 29.8 %, ($P < 0.001$), triglyceride by 4.5% ($P = 0.01$), in addition to an increased level of HDL-C in the intervention group by 9.5%, ($P = 0.01$). Also, in placebo group there were also a reduction on TC and non-HDL-C levels (6.7% and 10.3%, respectively) (both $P < 0.0001$). (Kasliwal et al., 2016)

3.3 Safety of Red Rice Yeast:

According to previous clinical trials, RYR can be used for hyperlipidemic patients with some exclusion criteria including hypertension, insulin-dependent type 1 and 2 diabetes, presence of neoplastic or hepatic diseases, chronic heart or renal failure, positive history or clinical signs of ischemic heart disease, disabilities like dementia or inability to cooperate, pregnancy or breast-feeding, chronic diseases and prior gastrointestinal surgical procedures, hormonal disorders, intake of lipid-lowering drugs and supplements. In all five articles that evaluate the safety of RYR treatment according to CK (creatine Kinase), AST (Aspartate aminotransferase), SCr (Serum creatinine), there were no significant adverse effects for RYR treatment during the study period.

Research was conducted in the Italian surveillance system to assess the safety profile of RYR by analyzing spontaneous reports of suspected adverse reactions shows that RYR had some adverse effects on patients with previous diseases and disorders. The research involved 1261 reports, the adverse reaction includes myalgia or elevation in creatine phosphokinase, rhabdomyolysis, liver injury, gastrointestinal reactions, cutaneous reactions, and other adverse reactions to RYR in 52 reports related to dietary supplements (Mazzanti et al., 2017). Also, in a randomized pilot trial to

compare the effect of Red yeast rice and simvastatin on dyslipidemia patients with low to moderate cardiovascular disorders, the results show that simvastatin had muscle fatigue symptoms, while RYR has no side effects and the serum lipid level in both groups is reduced. (Xu et al., 2017)

Table 1: Literature survey

No. article	title	Author/year	RYR-G	CG	RYR(Dosage)	TC		LDL-C		TG		HDL	
1	Low dose red yeast rice with monacolin K lowers LDL cholesterol and blood pressure in Japanese with mild dyslipidemia: A multicenter, randomized trial	Minamizuka et al. /2021	10	8	(200 mg/day) containing 2 mg monacolin K or diet therapy alone for 8 weeks	P=0.014*		P=0.030*		P=0.014*		P=0.082*	
2	Low dose red yeast rice with monacolin K lowers LDL cholesterol and blood pressure in Japanese with mild dyslipidemia: A multicenter, randomized trial	Mazza et al/2018	30	30	received 333 mg red yeast rice (10 mg monacolin K) for 12 weeks	CG	RYR-G	CG	RYR-G	CG	RYR-G	CG	RYR-G
						- 9.13%	- 18.32%	- 11.9%	- 23.88%	- 20.82%	- 24.51%	NM	NM
3	A low daily dose of 3 mg monacolin K from RYR reduces the concentration of LDL-C in a randomized, placebo-controlled intervention	Heinz et al /2016	72	70	3 mg monacolin k for 12 weeks	CG	RYR-G	CG	RYR-G	CG	RYR-G	CG	RYR-G
						-1.6%	-11.2%	-2.7%	-14.8%	NM	-5%	NM	NM
4	Red yeast rice lowers cholesterol in physicians - a double-blind, placebo controlled randomized trial	Verhoeven et al./2013	31	21	two capsules of RYR (5,025 mg of Monacolin K	CG	RYR-G	CG	RYR-G	CG	RYR-G	CG	RYR-G
						- 1.16%	14.62%	- 1.65%	22.17%	-4.97%	13.79 %	0.27%	- 2.99%
5	ESSENS dyslipidemia: A placebo-controlled, randomized study of a nutritional supplement containing red yeast rice in subjects with newly diagnosed dyslipidemia	Kasliwal et al /2016	92	80	400 mg of RYR twice a day	CG	RYR-G	CG	RYR-G	CG	RYR-G	CG	RYR-G
						-6.7%	-22.7%	-3.5%	-29%	NM	-4.5%	NM	+9.5%

*p-values indicate differences between the control and red yeast rice groups.

CG (Control Group), RYR-G (Red Yeast Rice -Group), TC (Total Cholesterol), LDL-C (Low-Density Lipoprotein -Cholesterol), TG(Triglycerides), HDL-C (High-Density Lipoprotein -Cholesterol), NM (Not Mention)

Conclusion:

In short, the active component in Red Yeast rice (monacolin K) can show a significant reduction of LDL-Cholesterol compared to the control group in hypercholesteremic at different concentrations (dosage). All five clinical trials suggest the use of RYR as a safe natural alternative for statin therapy, especially for hypercholesteremic patients with no history of chronic or acute organ dysfunction. The limitations of the five clinical trials were a short-duration study and a small sample size. Further clinical studies are required to prove the safety of RYR in long periods with a larger sample size.

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UNDER PEER REVIEW