Genetic Polymorphisms, LDL-C and sdLDL-C Help Optimize Coronary Heart Disease (CHD) Therapy

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Abstract

Background: Low-density lipoprotein cholesterol (LDL-C) and small, dense LDL-C (sdLDL-C) are important risk indicator of coronary heart disease (CHD), but their application in therapy monitoring of CHD is still far from being elucidated. Following the concept of precision medicine, we investigated whether the scientific medication based on medication-sensitive genes can reverse the LDL-C and sdLDL-C status in human bloodstream, so as to reveal the possibility of them as a monitoring indicator of CHD efficacy.

Methods: A prospective study of CHD cohort containing 208 Chinese CHD patients (158 males and 50 females) and 20 healthy people (14 males and 6 females) was recruited. LDL-C and its subfractions were detected before and after treatment. Polymorphism of medication-sensitive genes, including *SLCO1B1* (rs4149056, 521T>C), *CYP2C19*2* (rs4244285, c.681G>A), and *CYP2C19*3* (rs4986893, c.636G>A) were detected for medication guidance.

Results: Nearly half of Chinese CHD patients (47.60%, 99/208) had genetic polymorphisms with homozygous or heterozygous mutations within these three genes. LDL-1 and LDL-2, subfractions of LDL-C, had a 100% positive rate in CHD patients and healthy people. However, sdLDL-C components of LDL-5 to LDL-7 were only enrichment in CHD patients. Moreover, the mean amount of sdLDL-C subfractions in CHD patients was significantly higher than that in healthy people. Among 180 patients with treatment remission, 81.67% (n=147) of CHD patients had decreased LDL-C, while 61.67% (n=111) of patients had decreased sdLDL-C.

Conclusion: sdLDL-C has better accuracy on CHD screening than LDL-C, while LDL-C was more suitable for CHD therapy monitoring. Combined medication-sensitive genes polymorphism, LDL-C and sdLDL-C detection would optimize the treatment strategy for CHD patients.

Introduction

Coronary heart disease (CHD) is an important public health disease that causes death and disability [1]. Based on the data from the National Health and Nutrition Examination Survey (NHANES) 2011 to 2014, an estimated 16.5 million Americans ≥20 years of age had CHD, and approximately 720,000 new coronary events occurred in America in 2018 [1]. In China, more than 10 million people have been affected by this disease [2]. Low-density lipoprotein cholesterol (LDL-C) plays a pivotal role in the progression of CHD, as verified by genetic, pathology, observational, and intervention studies [3-6]. In 36,375 participants and a low 10-year risk cohort with long-term follow-up, LDL-C and non-HDL-C ≥160 mg/dL were independently associated with a 50% to 80% increased relative risk of cardiovascular disease (CVD) mortality [7]. The risk of CHD is accelerated by 10 to 20 years in men and 20 to 30 years in women with LDL-C levels ≥190 mg/dL [8], and the Adult Treatment Panel (ATP III) cholesterol guideline from 2001 [9] and the 2013 American College of Cardiology (ACC)\American Heart Association (AHA) guideline [10] recommend intensive treatment of patients with primary elevation of LDL-C [8, 11]. CHD treatment may include reducing the level of LDL-C to prevent recurrent cardiovascular events (CEs) [5],

and LDL-C has now largely replaced total cholesterol as a risk marker and the primary treatment target for hyperlipidemia [12].

LDL-C is a heterogeneous class of particles, and accumulating evidence suggests that different LDL subfractions vary in their risk profiles [13, 14]. Lipoprotein profiles consisting of primarily LDL-1 and LDL-2 subfractions have been designated as Pattern A, while profiles with predominantly small and dense subfractions (LDL-3 to LDL-7), known as small, dense LDLs (sdLDLs), have been designated as Pattern B [15]. Patients with the same LDL-C levels may have different cardiovascular risks, due to the difference on sdLDL-C subfractions [16-18]. In 808 participants from the Chinese Multiprovincial Cohort Study, sdLDL-C was independently associated with the progression of carotid atherosclerosis [19]. The study showed that sdLDL-C was the most effective predictor of residual risk of future CEs in those using statins and in high-risk coronary artery disease patients with diabetes or hypertriglyceridemia [20].

Although there are many reports about the excellent predictive performance of LDL-C and sdLDL-C for cardiovascular disease, the clinical application research especially in drug curative evaluation is still lacking; therefore, we wanted to assess patient status by LDL-C or sdLDL-C concentration change before and after using clopidogrel or statins to obtain more clinical application value, especially in Chinese CHD patients. A total of 208 CHD patients from the Quanzhou First Hospital Affiliated to Fujian Medical University (Fujian, China) were enrolled. CHD patients were assessed for LDL-C and sdLDL-C before and after treatment with statins or clopidogrel until the disease was alleviated.

To improve the treatment effect, genetic testing before medication administration was recommended to patients [21-23]. Simvastatin is the most common prescription medication for cholesterol reduction, but a single nucleotide polymorphism (SNP), rs4149056 T>C, in *SLCO1B1* increases systemic exposure to simvastatin and the risk of muscle toxicity [21]. Individuals carrying the *CYP2C19*2* allele have impaired pharmacodynamic responses to clopidogrel [22]. *CYP2C19*2* and *CYP2C19*3* can be genetically screened, and appropriate dose adjustments can be made on the basis of a patient's *CYP2C19* genotype [23]. Therefore, we intended to perform pretreatment screening of *SLCO1B1* (rs4149056, 521T>C), *CYP2C19*2* (rs4244285, c.681G>A), and *CYP2C19*3* (rs4986893, c.636G>A) with an Agena Bioscience MassARRAY system, which is an advanced detection system based on MALDI-TOF MS technology, with superfunction to detect dozens of gene loci in one sample [24]. Finally, the polymorphisms of these three genes were analyzed to help implement more effective treatment, and LDL-C and sdLDL-C data before and after treatment were analyzed. Overall, the clinical values of multigene testing, LDL-C or sdLDL-C were evaluated to acquire the best therapeutic effect for CHD patients. The flow chart is shown in Figure 1A.

Materials And Methods

Patients and sample collection

Subjects were enrolled from the Quanzhou First Hospital Affiliated to Fujian Medical University (Fujian, China) from Jan 2018 to May 2020. There were a total of 228 subjects, including 208 CHD patients (158 males and 50 females) with a mean age of 64.48 and 20 healthy people (14 males and 6 females) with a mean age of 45.05 (Table 1). Peripheral blood samples (0.5 mL each) were extracted from subjects and centrifuged at 800 × g for 10 minutes to obtain supernatant plasma samples (0.2 mL each) for LDL-C detection and peripheral blood cell sediment for MassARRAY SNP detection. Under the guidance of the results of SNP, all CHD patients were given precise medication (mainly statins) according to the dosage recommended in Technical Guidelines for Gene Detection of Drug Metabolic Enzymes and Drug Targets (2015, China).

LDL-C, sdLDL-C and SNP detection

The detection of LDL-C, sdLDL-C and medication-sensitive genes, including SLCO1B1 (rs4149056, 521T>C), CYP2C19*2 (rs4244285, c.681G>A), and CYP2C19*3 (rs4986893, c.636G>A) were performed by a third-party medical diagnostics company with clinical laboratory qualification. The technique details were listed in Supplementary Materials.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 statistical software (GraphPad Software, Inc., San Diego, CA, USA). $P<0.05$ indicated a statistically significant difference.

Results

Multitudinous gene polymorphisms associated with drug efficacy in Chinese CHD patients.

Statins, clopidogrel and other drugs were used for CHD treatment in the clinic. To ensure good therapeutic effects, genetic testing was recommended for patients before remedy with statins or clopidogrel [21-23]. In previous studies, *SLCO1B1* (rs4149056, 521T>C) increased systemic exposure to simvastatin and had a risk of muscle toxicity, *CYP2C19*2* and *CYP2C19*3* could affect appropriate drug dose adjustment [21, 23], and gene loci *SLCO1B1* (rs4149056, 521T>C), *CYP2C19*2* (rs4244285, c.681G>A), and *CYP2C19*3* (rs4986893, c.636G>A) were detected in this study by MassARRAY before treatment.

After testing, 47.60% (99/208) of patients had genetic polymorphisms of homozygous or heterozygous mutations; in specific, *SLCO1B1* (521T>C) TT, TC, and CC were identified in 80.29%, 19.23%, and 0.48% of patients, respectively; *CYP2C19*2* (681G>A) GG, GA, and AA were identified in 60.10%, 34.13%, and 5.77% of patients, respectively, while *CYP2C19*3* (636G>A) GG, GA, and AA were identified in 92.31%, 7.69%, and 0.00% of patients, respectively (Figure 1B). The results showed that these three genes had multitudinous

polymorphisms in Chinese CHD patients and further affirmed the significance of polymorphism detection before statin or clopidogrel therapy.

CHD patients had higher expression and more types of sdLDL-C subfractions.

For subfractions of LDL-C and sdLDL-C that were related to CVD [16-18], the precise subfraction expression levels of LDL-C and sdLDL-C in CHD patients and healthy people were detected using a Quantimetrix Lipoprint system. After analysis, the typical lipoprotein subfraction images showed that LDL-1 and LDL-2, known as Pattern A, collectively existed in the plasma of healthy people and CHD patients (Figure 2A), while CHD patients had extra LDL-3 to LDL-7, known as Pattern B or sdLDL-C (Figure 2B). To ensure robust results, samples from 228 subjects were analyzed, including 208 CHD patients and 20 healthy people (Table 1).

The results revealed that the detection rates of LDL-1 to LDL-7 subfractions in CHD patients were 100%, 100%, 99.04%, 94.71%, 62.98%, 18.75%, and 4.33%, respectively, while those in healthy people were 100%, 100%, 95%, 25%, 0%, 0%, and 0%, respectively (Figure 2C). Pattern A had a 100% detection rate in CHD patients and healthy people, while sdLDL-C subfractions were more abundant in CHD patients than in healthy people (Figure 2C). This result was also consistent with previous studies, and sdLDL-C was a high risk factor for CHD [17]. Then, the mean amounts of subfractions were calculated; LDL-1 to LDL-7 in CHD patients were 28.26 mg/dL, 31.47 mg/dL, 18.14 mg/dL, 9.13 mg/dL, 2.74 mg/dL, 0.49 mg/dL, and 0.28 mg/dL, while these subfractions in healthy people were 45.05 mg/dL, 23.70 mg/dL, 31.15 mg/dL, 22.8 mg/dL, 5.7 mg/dL, 0.35 mg/dL, 0 mg/dL, 0 mg/dL, and 0 mg/dL, respectively (Figure 2D and Table 1). CHD patients had higher expression of sdLDL-C subfractions than healthy people.

Table 1
Participant information (prior to treatment)

	CHD patients (Mean ± SD)			Healthy people (Mean ± SD)		
	Male	Female	All	Male	Female	All
	n = 158	*n* = 50	*n* = 208	*n* = 14	*n* = 6	*n* = 20
Age (year)	62.46 ± 12.08	70.86 ±7.90	64.48 ± 11.76	45.57 ± 9.50	43.83 ± 6.27	45.05 ± 8.53
Body mass index (kg/m^2)	24.06 ± 2.89	24.53 ± 2.99	24.17 ± 2.91	24.17 ± 1.97	22.60 ± 2.77	23.70 ± 2.29
LDL-1（mg/dl）	28.06 ± 14.91	28.88 ± 15.68	28.26 ± 15.06	31.0 ± 14.33	31.5 ± 12.57	31.15 ± 13.50
LDL-2（mg/dl）	31.85 ± 14.15	30.26 ± 13.05	31.47 ± 13.88	23.0 ± 7.42	22.33 ± 6.41	22.8 ± 6.97
LDL-3（mg/dl）	17.94 ± 9.60	18.76 ± 10.92	18.14 ± 9.92	5.64 ± 3.05	5.83 ± 2.04	5.7 ± 2.74
LDL-4（mg/dl）	8.92 ± 7.78	9.78 ± 9.10	9.13 ± 8.10	0.5 ± 0.76	0	0.35 ± 0.67
LDL-5（mg/dl）	2.86 ± 4.15	2.34 ± 3.18	2.74 ± 3.94	0	0	0
LDL-6（mg/dl）	0.59 ± 2.35	0.18 ± 0.48	0.49 ± 2.07	0	0	0
LDL-7（mg/dl）	0.35 ± 2.52	0.06 ± 0.42	0.28 ± 2.21	0	0	0
Total cholesterol (mmol/l)	5.02 ± 1.53	5.47 ± 1.72	5.13 ± 1.59	3.85 ± 0.71	3.97 ± 0.69	3.88 ± 0.69
Total triglycerides (mmol/l)	1.45 ± 0.94	1.63 ± 0.93	1.50 ± 0.94	1.27 ± 0.85	1.09 ± 0.31	1.21 ±0.72
Plasma HDL-C (mmol/l)	1.09 ± 0.46	1.19 ± 0.24	1.11 ± 0.42	1.35 ± 0.25	1.40 ± 0.08	1.36 ± 0.21
Plasma LDL-C (mmol/l)	3.21 ± 1.28	3.51 ± 1.50	3.28 ± 1.34	2.26 ± 0.68	2.31 ± 0.63	2.28 ± 0.65
Apo A1 (g/l)	1.25 ± 0.25	1.34 ± 0.21	1.27 ± 0.24	1.28 ± 0.08	1.25 ± 0.10	1.27 ± 0.09
Apo B (g/l)	1.04 ± 0.33	1.13 ± 0.37	1.06 ± 0.34	0.69 ± 0.16	0.81 ± 0.24	0.73 ± 0.19

LDL-C had better properties for CHD monitoring.

The clinical effect of LDL-C and sdLDL-C was then studied on CHD patient monitoring. A total of 208 CHD patients (158 males and 50 females) were enrolled, and their disease was alleviated after treatment. In total, 169 patients (81.25%) and 126 patients (60.58%) exhibited LDL-C and sdLDL-C decreases (Figure 3A). All 208 CHD patients were evaluated for LDL-C and sdLDL-C before treatment; after treatment and disease remission, 180 CHD patients (134 males and 46 females) underwent detection once, and the percentage of patients with an LDL-C decrease was 81.67% (n=147), while the percentage of patients with an sdLDL-C decrease was 61.67% (n=111) (Figure 3A). Twenty patients (17 males and 3 females) underwent posttreatment detection twice; the percentages with LDL-C and sdLDL-C decreases were 80.00% (n=16) and 55.00% (n=11), respectively (Figure 3A).

To observe the dynamic changes of LDL-C and sdLDL-C in the process of disease remission, 8 CHD patients (7 males and 1 female) who underwent detection four times (one time before treatment and three times after treatment) were analyzed (Figure 3B). As shown in from Figure 3A, the percentages of patients with LDL-C and sdLDL-C decreases were 75.00% (n=6) and 50.00% (n=4), respectively. However, the expression changes of LDL-C and sdLDL-C in the same patient were not exactly the same. For instance, the expression levels of LDL-C and sdLDL-C in patient 6 were both significantly decreased and remained relatively stable, with low expression in follow-up monitoring, while patient 5 had low expression sdLDL-C after therapy but had higher expression of LDL-C, though anesis of the CHD occurred after treatment (Figure 3C). Overall, the expression of LDL-C was more consistent with the disease course of the patient than sdLDL-C.

LDL-C had a better monitoring effect than other clinical biomarkers.

To better understand the monitoring utility of LDL-C and sdLDL-C in CHD patients, they were compared with other clinical biomarkers, such as total cholesterol, total triglycerides, plasma HDL-C, plasma LDL-C, Apo A1, and Apo B. Forty-seven CHD patients were chosen from the 208 patients, and all of these biomarkers were detected before and after treatment. When CHD anesis occurred, the expression levels of total cholesterol, total triglycerides, plasma LDL-C, Apo A1, Apo B, LDL-C and sdLDL-C were decreased, and plasma HDL-C was possibly increased.

Among the 47 CHD patients (39 males and 8 females), the percentages of those with decreased total cholesterol, total triglycerides, plasma LDL-C, Apo A1, Apo B, LDL-C, and sdLDL-C and increased plasma HDL-C were 82.98%, 40.43%, 80.85%, 53.19%, 65.96%, 76.60%, 65.96%, and 25.53%, respectively (Figure 3D). Although 82.98% of the CHD patients had decreased total cholesterol, total cholesterol is composed of LDL-C and HDL-C and thus was not clinically significant (Figure 3D). LDL-C was a better biomarker for CHD treatment monitoring than sdLDL-C and other clinical biomarkers.

Conclusion. sdLDL-C was more suitable for CHD screening than LDL-C, while LDL-C was more suitable for CHD monitoring than sdLDL-C. Combined medication-related gene polymorphism, LDL-C and sdLDL-C detection would better optimize the treatment strategy for Chinese CHD patients.

Discussion

Statins are currently effective in the treatment of CHD and lowering blood lipids [26], and clopidogrel has been recommended to treat CHD in current clinical practice guidelines [27]. Before use, *SLCO1B1* (rs4149056, 521T>C), *CYP2C19*2* (rs4244285, c.681G>A), and *CYP2C19*3* (rs4986893, c.636G>A) were detected to ensure the best treatment was administered [21-23]. In this study, the SNPs of these genes were detected by a Agena Bioscience MassARRAY system, which had the advantages of detecting dozens of gene loci in one sample [24], requiring fewer samples, having a lower cost, and obtaining results in a shorter time than the Sanger sequencing method [28]. The results showed that there were three genotypes associated with *SLCO1B1* (521TT, 521TC, 521CC), *CYP2C19*2* (681GG, 681GA, 681AA) and two genotypes associated with *CYP2C19*3* (636GG, 636GA) in these 208 CHD patients (Figure 1B). A total of 47.60% patients had homozygous or heterozygous mutations (Figure 2C), and polymorphisms detection before statin or clopidogrel therapy could lead to better treatment strategies for Chinese CHD patients.

Numerous studies have shown that LDL-C and sdLDL-C were related to CEs [18, 20]. LDL-C subfractions were detected prior to treatment in CHD patients and healthy people, and we identified the presence of LDL-5 C, LDL-6 C, and LDL-7 C in CHD patients, but not in healthy people (Figure 2C and Figure 2D, Table 1). The mean amounts of sdLDL-C subfractions were calculated; CHD patients also had higher expression of sdLDL-C subfractions than healthy people (Figure 2D). These results suggested that sdLDL-C (LDL-3 C to LDL-7 C) was a high-risk factor for CHD; thus, sdLDL-C detection had greater clinical significance in CHD screening. Our results were consistent with previous studies [16-18].

Conclusions

In the meantime, the monitoring effect of LDL-C and sdLDL-C was affirmed; after treatment and remission, CHD patients with LDL-C and sdLDL-C decreases attained 81.25% and 60.58% (Figure 3A). Although the change curves of LDL-C and sdLDL-C expression were not necessarily consistent in one patient (Figure 3C), in general, the coincidence rate of LDL-C in the disease state was greater than that of sdLDL-C. Other clinical biomarkers, total cholesterol, total triglycerides, plasma HDL-C, plasma LDLC, Apo A1, and Apo B versus LDL-C and sdLDL-C, and ultimately LDL-C were also better biomarkers for CHD treatment monitoring (Figure 3D).

Declarations

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Authors' contributions:

Ruozhu Dai, Xinjun Wang and Xiaoyu Zhao contributed to the study conception and experimental design. Huilin Zhuo contributed to the clinical sample collection, sampled detection and data analysis. Xiaoyu Zhao, Xinjun Wang and Wei Wang contributed to data analysis, manuscript writing and revision. All authors critically revised the manuscript, gave final approval and agreed to be accountable for all aspects of the work, ensuring its integrity and accuracy.

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Availability of data and materials:

All data generated or analysed during this study are included in this published article and its supplementary information files.

Ethics approval and consent to participate:

All experimental protocols in this study was approved by the ethics committee of Quanzhou First Hospital Affiliated with Fujian Medical University. All patients provided written informed consent for this study. All methods in this study carried out in accordance with the *Declaration of Helsinki*.

Consent for publication:

Informed consent was obtained from all participants for publication.

Conflicting Interests:

The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Figures

Figure 1

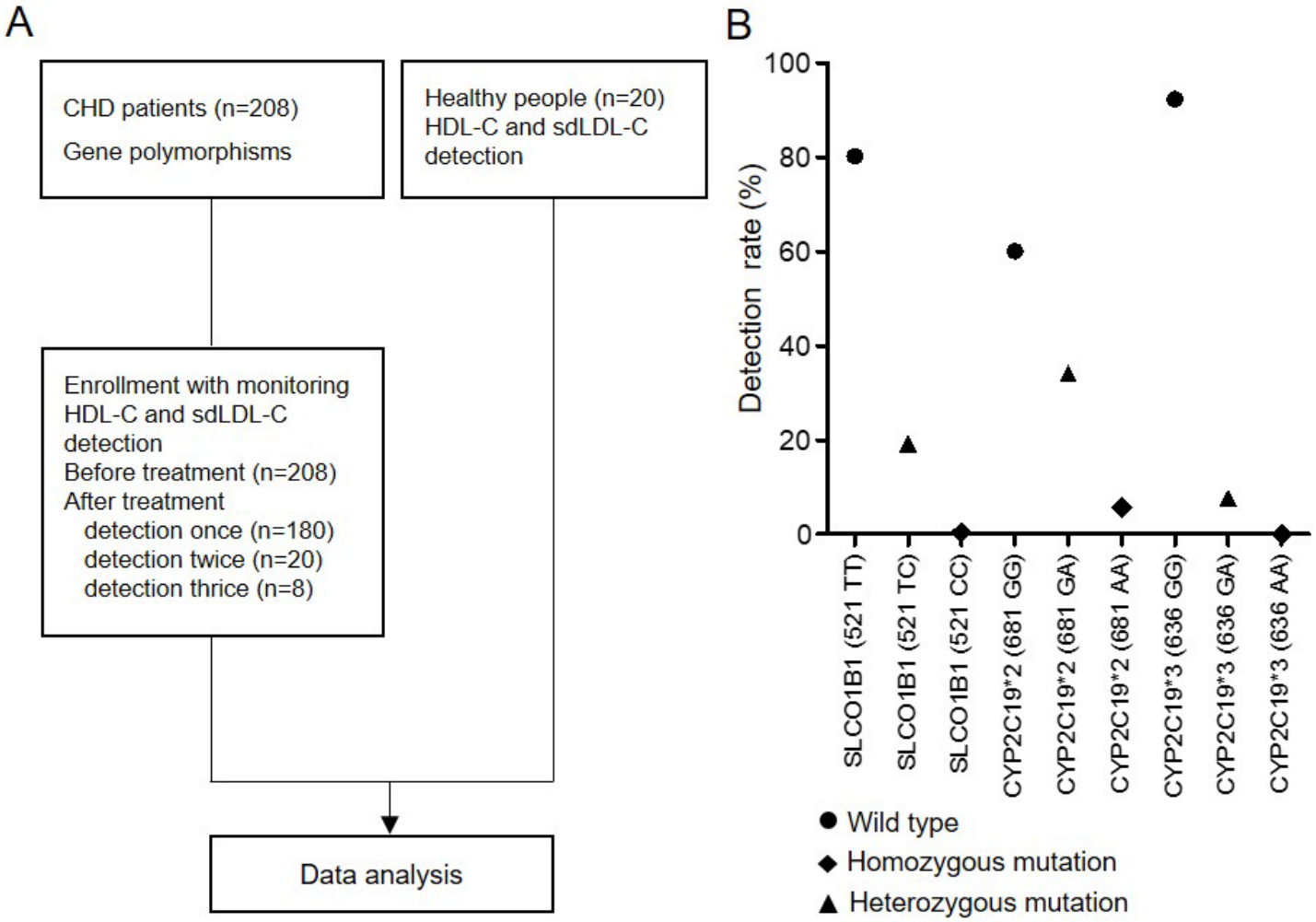

Figure 1

(A) Flow Chart. (B) SLCO1B1 (rs4149056, 521T>C), CYP2C19*2 (rs4244285, c.681G>A), and CYP2C19*3 (rs4986893, c.636G>A) testing in 208 CHD patients.

Figure 2

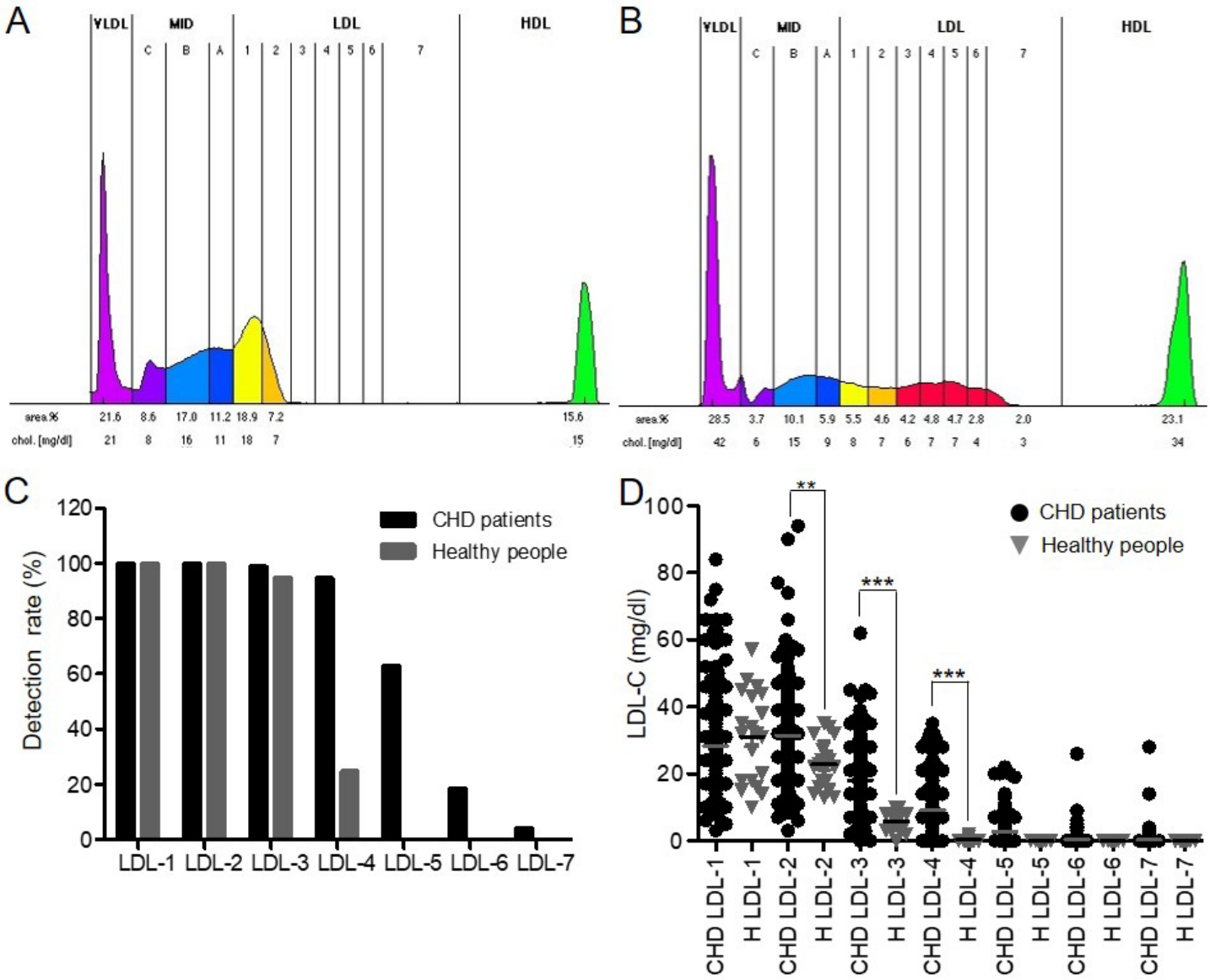

Figure 2

LDL-C subfraction detection. (A) LDL-C subfraction detection in healthy people. (B) LDL-C subfraction detection in CHD patients. (C) Detection rate of LDL-1 to LDL-7 subfractions in CHD patients (n=208) and healthy people (n=20). (D) Mean amount of LDL-1 to LDL-7 subfractions in CHD patients (n=208) and healthy people (n=20), ANOVA, ** $P < 0.01$, *** $P < 0.001$.

Figure 3

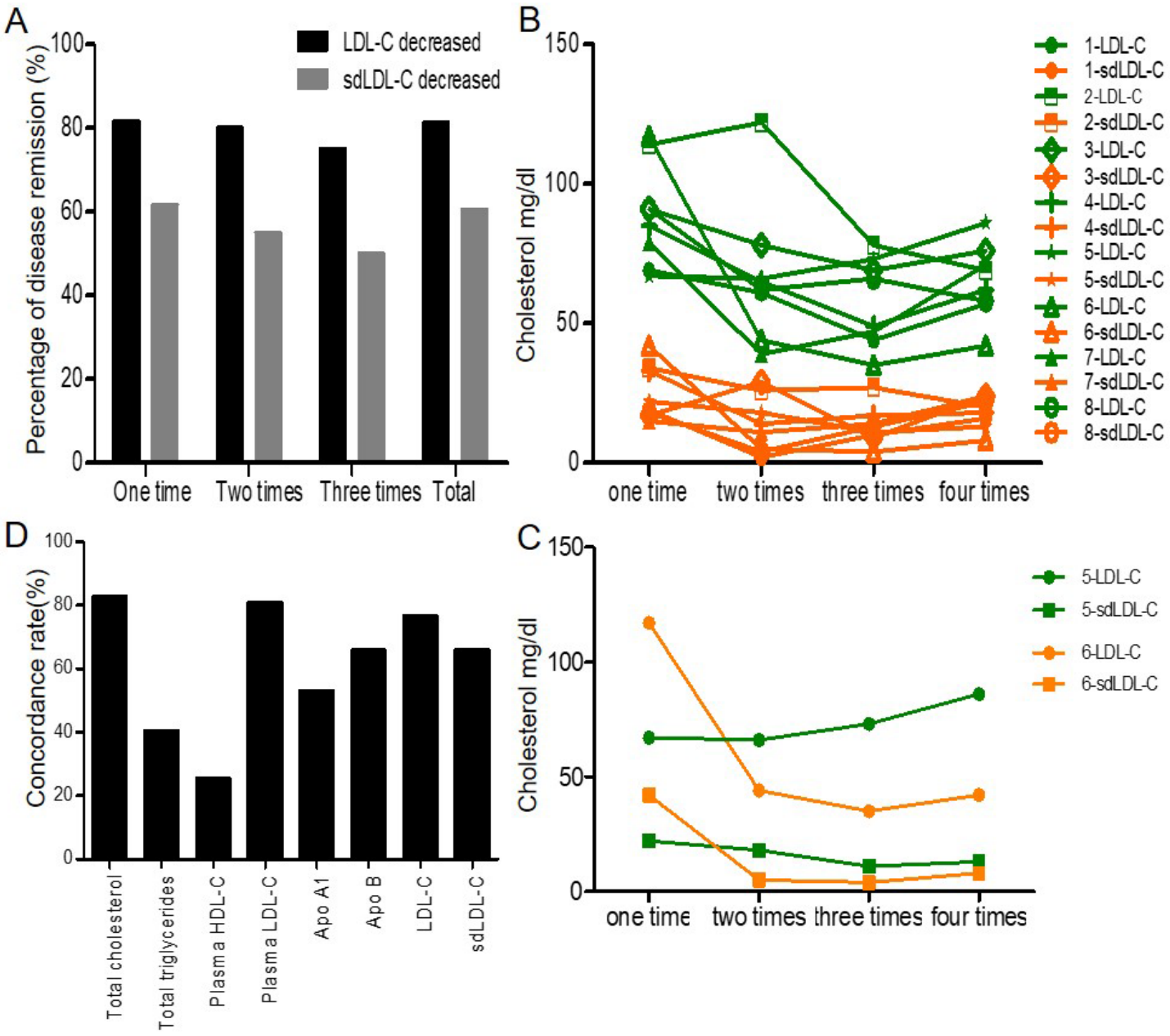

Figure 3

LDL-C and sdLDL-C monitoring in CHD patients. (A) CHD patients underwent detection after treatment: one time (n=180, 134 males and 46 females), two times (n=20, 17 males and 3 females), three times (n=8, 7 males and 1 female), total (n=208, 158 males and 50 females). (B) LDL-C was detected in 8 CHD patients (7 males and 1 female) four times, including one time before treatment and three times after treatment. The numbers 1 to 8 represent patients 1 to 8. (C) LDL-C was detected in CHD patient 5 and CHD patient 6 four times, including one time before treatment and three times after treatment. (D)

Concordance rates of 47 CHD patients (39 males and 8 females) who underwent detection of total cholesterol, total triglycerides, plasma HDL-C, plasma LDL-C, Apo A1, Apo B, LDL-C and sdLDL-C.

Supplementary Files

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