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SHORT COMMUNICATION

Susan E. Near · Jian Wang · Robert A. Hegele

Single nucleotide polymorphisms of the very low density lipoprotein receptor (*VLDLR*) gene

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Abstract The very low density lipoprotein receptor (VLDLR) has a potentially important role in lipoprotein metabolism and Alzheimer's disease. We developed amplification primers for most of the coding region and 3′-untranslated region of *VLDLR* and used sequencing of genomic DNA to examine these regions of *VLDLR* in subjects with familial combined hyperlipidemia and in normal controls. We identified ten novel single nucleotide polymorphisms (SNPs) for *VLDLR*. We also found one rare coding sequence variant, S>R153, in a subject with familial combined hyperlipidemia, which was absent from 2360 normal alleles. The identification of intron–exon boundaries, amplification primers, and SNPs provides tools to investigate *VLDLR* for genetic association and linkage studies.

Key words Lipoproteins · Metabolism · Alzheimer's disease · Atherosclerosis · Genomic DNA · Sequencing · Complex traits

Introduction

The human very low density lipoprotein receptor (VLDLR; OMIM 192977) appears to play a key role in triglyceride metabolism. The *VLDLR* gene on 9p24 is highly expressed in heart, muscle, and adipose tissues, which are active in fatty acid metabolism, but is not expressed in liver (Oka et al. 1994). The *VLDLR* gene contains 19 exons spanning ~40 kb and its intron–exon organization is almost identical to that of the low density lipoprotein receptor (LDLR; OMIM 143890). In contrast to *LDLR*, however, no human mutations or single nucleotide polymorphisms (SNPs) have yet been reported in *VLDLR*. A polymorphic triplet repeat in *VLDLR* has been reported to be associated with Alzheimer's disease (Okuizumi et al. 1995). Because of the possible association with Alzheimer's disease and because of its potential role in lipoprotein metabolism, we developed amplification primers to sequence the genomic DNA of most of the coding region and 3′-untranslated region of *VLDLR* in subjects with familial combined hyperlipidemia and in normal control subjects.

S.E. Near · J. Wang · R.A. Hegele (✉)
Blackburn Cardiovascular Genetics Laboratory, John P. Robarts Research Institute, 406-100 Perth Drive, London, ON N6A 5K8, Canada
Tel. +1-519-663-3461; Fax +1-519-663-3789
e-mail: robert.hegele@rri.on.ca

Subjects and methods

Study subjects

We screened genomic DNA from samples taken from 12 unrelated subjects with familial combined hyperlipoproteinemia (defined as plasma triglyceride and LDL cholesterol concentrations both in excess of the 95th percentile for age and sex, with at least one other affected relative) and 6 unrelated normolipidemic subjects. Samples from a DNA archive of 20 unaffected normal Caucasian subjects were screened to determine allele frequencies. For the 540C>T SNP, 1180 normolipidemic subjects from six ethnic groups were screened. The study was approved by the Ethics Review Panel, University of Western Ontario.

Screening the *VLDLR* gene for DNA variants

Insufficient published sequence data were available to develop primers that would span at least 50 bp of DNA within the introns adjacent to the intron–exon boundaries. We used an established method to identify most intron–exon boundaries of *VLDLR* (Table 1) and at least 100 bp of flanking intron sequence (Wang et al. 2000). We used this information to develop primers to amplify coding regions of *VLDLR* and intron–exon boundaries from genomic DNA.

Table 1. Intron–exon boundaries for *VLDLR* gene

Exon	5′ Boundary	3′ Boundary
2		CTGTGgtaagtaaagagtttgatgac
3	ccttcaaataaacgtttgtagTAAAG	GTGCCgtgagtgtaacttgctttggc
4	agacgtgtatatcatcaacagATATG	CTGTGgtaagaagatcagtgttgagt
5	gggcatcctctctcttaatagGCAAT	CTGTCgtaagtagctttctagcatgg
6	tggtgtttcctccctttgtagCCTCT	AAATGgtaagggtttcttcttgttgg
7	cttctttgtttctctttgtagTCAAT	GTGTCgtaagtgtacttgttgttcaa
8	actactacatttttattccagATATA	TGGAGgtgagtctaagaagaaaacct
9	taattctgatttccctcccagATATT	AGTAGgtaaatgaacttggactggta
10	acccagacttccatcttgcagGCAAA	TTCAGgtaactttcagttccttttgt
11	ttgtgacctattctgtttcagTGCCT	TCTGGgtttgtagtctgttttccatc
12	ttctcatttaatttttcacagCTTTG	ACTTGgtatgtatgttcttccttctc
13	gacaattcttttcctacctagACCTT	TTGAGgtaagatgtgtctacatcaaa
14	aattgtgggcttctgttttagGATCG	ATCAGgtaccgtggagaagcacagtc
15	ggtatttttttttcctgactagGTAAA	TCAAAgtaaggcattttgtgtttcaa
16	ttcttggtttttataattcagGTACT	TGGAGgtattgagttcagtactgcaa
17	attccttttattcctctgtagGGATC	TCTCTgtaagtagatttcctacaagt
18	ctaccctctgatttttttcagTGCTC	CAGCAgtaagtcagctttgtgtcttt
19	tatacttcttcttttccacagATATC	

Table 2. Amplification primers for *VLDLR* gene

Exon	Primer sequence	Product size (bp)
3	Forward: 5′-CTGGATATTGGCAGTTGAGTG Reverse: 5′-CCCTTGGAGTCAATTGACCTA	398
4	Forward: 5′-TGCATTGATCAGTTCTGAGGC Reverse: 5′-GTCAATGTCAGTGCGTCTTCA	275
5	Forward: 5′-GACAAGAATCTTGAACGGAC Reverse: 5′-GTTGATACAGGGAAGAGAAC	482
6	Forward: 5′-GAGGACAACTGTCGTAAGTAG Reverse: 5′-CATTCTCCACTTCTGCACTTG	364
7	Forward: 5′-GATGGTTCCGATGAAGTCAAC Reverse: 5′-CTGGATTGTGTCAAACTTCCAG	290
8	Forward: 5′-GGTCTTAGACAAATCGTGGG Reverse: 5′-CTATCCTTTCCATCACCTG	293
9	Forward: 5′-AGGTATAGGAGCAGCAAGAC Reverse: 5′-GAGGTTAGCAGCATTGTTCTC	294
10	Forward: 5′-TCCAGAACAGATACTACTGAGG Reverse: 5′-CCCAAGTGACAATGACTTATGTC	357
11	Forward: 5′-TAGGATCAGTAAGCAGCATGG Reverse: 5′-GACCTACACAGATACCATTCC	434
12	Forward: 5′-GTCACTAGAGAATGCCTTGAG Reverse: 5′-GTCCAGAATTCACCATGGAG	314
13	Forward: 5′-CAGAGTAGTAGTGGCTTGTC Reverse: 5′-TCTCAAGCCATGTTCAGCTGC	300
14	Forward: 5′-AGACACTAAGTCCCAGAAGC Reverse: 5′-CAAAGAGCTACTGACATCCA	407
15	Forward: 5′-GACTCAGGTCTTCAACATGTAG Reverse: 5′-GGTATGTTGACACTTTAGTCTCC	454
16	Forward: 5′-GTACAACTTAGTTCAGCAGTGG Reverse: 5′-CTGTAGATACAAAGAGTGTGGC	280
17	Forward: 5′-CAGAGTTGTTCCTGGTGTTCA Reverse: 5′-AGCGAACGTAATTCAGTGTCA	354
18	Forward: 5′-GGCTGAACTTGTTTCAAGCTCC Reverse: 5′-GTGTTTGGTAACTCCATCATCC	455
19	Forward: 5′-CTAGAGTTGCCATCAGTGAGTG Reverse: 5′-CAGATACTCGGTAACCACATCC	317

Primer sequences are shown in Table 2. Primers were designed to anneal at a single temperature, which allowed for use of a single amplification apparatus. Amplification conditions were 94°C for 5 min, followed by 30 cycles of 30 s each at 94°C, 58°C, and 72°C, and ending with a single 10-min extension step at 72°C.

Genotyping of *VLDLR* SNPs

Six common *VLDLR* SNPs and the rare 540C>T SNP were found to alter recognition sites for restriction endonucleases (Table 3), which became the basis for genotyping. The remaining four common *VLDLR* SNPs did not alter

Table 3. *VLDLR* gene SNPs

Position	Nucleotide change	Detection method	Frequency
Exon 5 (S>R153)	540C>T	*Bbv*I digestion	Absent from 2360 normal alleles
−3 nt I8/e9 boundary	C>T	*Bfa*I digestion	T: 0.25
+27 nt e9/I9 boundary	G>A	*Mun*I digestion	A: 0.25
−17 nt I9/e10 boundary	A>T	*Dde*I digestion	T: 0.25
Exon 14 (Q662)	2067A>G	Direct sequencing	2067G: 0.25
−75 nt I15/e16 boundary	A>G	*Nla*III digestion	G: 0.30
−67 nt I15/e16 boundary	T>C	*Scr*FI digestion	C: 0.05
−30 nt I15/e16 boundary	T>C	*Mse*I digestion	C: 0.05
−65 nt I16/e17 boundary	T>C	Direct sequencing	C: 0.05
+8 nt e17/I17 boundary	G>T	Direct sequencing	T: 0.35
3′-UTR	2801G>A	Direct sequencing	A: 0.05

nt, Nucleotide; UTR, untranslated region

restriction endonuclease recognition sites (Table 3) and these were genotyped by using direct genomic DNA sequencing.

The *VLDLR* SNP that was located 3 nucleotides (nt) upstream of the intron 8/exon 9 boundary was genotyped by amplification of a 154-bp fragment with primers F5′-CAGGTGATGGGAAAGGATAGTATGTAC-3′ and R5′-ACGACTACATTCACACTTGTAACCGC-3′. The amplification conditions were 94°C for 5 min, followed by 30 cycles of 30 s each at 94°C, 55°C, and 72°C, and ending with a single 10-min extension step at 72°C. Digestion of the [−3]C allele with *Bfa*I produced two fragments of 27 and 127 bp, whereas digestion of the [−3]T allele produced three fragments of 27, 42, and 85 bp. These fragments were resolved by electrophoresis in 10% polyacrylamide gels.

The *VLDLR* SNP that was located 27 nt downstream from the exon 9/intron 9 boundary was genotyped by amplification of a 147-bp fragment with primers F5′-TGAACTTGGACTGGTATGGCA-3′ and R5′-GCTATTTGACCTATACTGCTG-3′. The amplification conditions were 94°C for 5 min, followed by 30 cycles of 30 s each at 94°C, 53°C, and 72°C, and ending with a single 10-minute extension step at 72°C. Digestion of the [+27]G allele with *Mun*I produced a single fragment of 147 bp, whereas digestion of the [+27]A allele produced two fragments of 20 and 127 bp. These fragments were resolved after electrophoresis in 10% polyacrylamide gels.

The *VLDLR* SNP that was located 17 nt upstream of the intron 9/exon 10 boundary was genotyped by amplification of a 154-bp fragment with primers F5′-GGCAGGAACTCCAGAACAGATACTACTGAGG-3′ and R5′-CTTGGCTCTTTGCCTGCAAGATGGAACTC-3′. The amplification conditions were 94°C for 5 min, followed by 30 cycles of 30 s each at 94°C, 58°C, and 72°C, and ending with a single 10-minute extension step at 72°C. Digestion of the [−17]A allele with *Dde*I produced two fragments of 26 and 128 bp, whereas digestion of the [−17]T allele produced three fragments of 26, 98, and 30 bp. These fragments were resolved by electrophoresis in 10% polyacrylamide gels.

The *VLDLR* SNP that was located 75 nt upstream of the intron 15/exon 16 boundary was genotyped by amplification of a 280-bp fragment with primers F5′-GTACAACTTAGTTCAGCAGTGG-3′ and R5′-CTGTAGATACAAAGAGTGTGGC-3′. The amplification conditions were 94°C for 5 min, followed by 30 cycles of 30 s each at 94°C, 58°C, and 72°C, and ending with a single 10-minute extension step at 72°C. Digesting the [−75]A allele with *Nla*III produced three fragments of 62, 28, and 190 bp, whereas digestion of the [−75]G allele produced two fragments of 90 and 190 bp. These fragments were resolved by electrophoresis in 10% polyacrylamide gels.

The *VLDLR* SNP that was located 30 nt upstream of the intron 15/exon 16 boundary was genotyped by amplification of a 280-bp fragment with primers F5′-GTACAACTTAGTTCAGCAGTGG-3′ and R5′-CTGTAGATACAAAGAGTGTGGC-3′. The amplification conditions were 94°C for 5 min, followed by 30 cycles of 30 s each at 94°C, 58°C, and 72°C, and ending with a single 10-minute extension step at 72°C. Digestion of the [−30]T allele with *Mse*I produced four fragments of 42, 62, 139, and 37 bp, whereas digestion of the [−30]C allele produced three fragments of 42, 201, and 37 bp. These fragments were resolved by electrophoresis in 10% polyacrylamide gels.

The *VLDLR* SNP that was located 67 nt upstream of the intron 15/exon 16 boundary was genotyped by amplification of a 280-bp fragment with primers F5′-GTACAACTTAGTTCAGCAGTGG-3′ and R5′-CTGTAGATACAAAGAGTGTGGC-3′. The amplification conditions were 94°C for 5 min, followed by 30 cycles of 30 s each at 94°C, 58°C, and 72°C, and ending with a single 10-minute extension step at 72°C. Digesting the [−67]T allele with *Scr*FI produced five fragments of 68, 30, 11, 102, and 69 bp, whereas digestion of the [−67]C allele produced four fragments of 98, 11, 102, and 69 bp. These fragments were resolved by electrophoresis in 10% polyacrylamide gels.

The *VLDLR* 540C>T SNP that resulted in a missense mutation at residue 153 (S>R153) was genotyped by amplification of a 483-bp fragment with primers F5′-GACAAGAATCTTGAACGGAC-3′ and R5′-GTTGATACAGGGAAGAGAAC-3′. The amplification conditions were 94°C for 5 min, followed by 30 cycles of 30 s each at 94°C, 58°C, and 72°C, and ending with a single 10-minute extension step at 72°C. Digestion of the [540]C allele with *Bbv*I produced four fragments of 96, 68, 62, and 257 bp, whereas digestion of the [540]T allele produced three frag-

ments of 96, 130, and 257 bp. These fragments were resolved after electrophoresis in 10% polyacrylamide gels.

Statistical analysis

SAS version 6.12 (SAS Institute, Cary, NC, USA) was used for statistical analyses. Allele frequencies were determined from electrophoretogram tracings of genomic DNA sequence, except for two SNPs assayed by using restriction digestion. Chi-square analysis tested the deviation of genotype frequencies from Hardy-Weinberg predictions. The nominal level of significance for statistical analyses was $P < 0.05$.

Results

Identification of SNPs

Most intron–exon boundaries were identified (Table 1) and amplification primers were developed (Table 2). Sequencing found ten common SNPs in subjects with familial combined hyperlipidemia and in normal controls. In addition, a rare coding SNP (540C>T; S>R153) was found in a subject with familial combined hyperlipidemia.

SNP frequencies

The observed genotype frequencies of all SNPs did not deviate from the predictions of the Hardy-Weinberg equation. The allele frequencies are shown in Table 3. The *VLDLR* 540C>T SNP was absent from 2360 alleles taken from normal subjects.

Discussion

We report intron–exon boundaries and definition of primer sets to amplify the coding sequences of the *VLDLR* gene and the use of these amplification primers for genomic DNA sequencing, which has resulted in the identification of ten novel SNPs and a rare coding region variant found only in a single subject to date. Because we did not examine the *VLDLR* promoter regions and exon 1, we cannot absolutely rule out the presence of such SNPs. The reagents described in this report could be helpful to screen the *VLDLR* gene when it becomes a candidate for phenotypes, either through positional cloning, analogy with other phenotypes, or expression data showing changes in response to particular experimental interventions. The reported *VLDLR* SNPs could also be helpful in linkage mapping and for association studies.

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