J Hum Genet (2001) 46:423–425

SHORT COMMUNICATION

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Single nucleotide polymorphisms of *RXRA* encoding retinoid X receptor alpha

Received: February 21, 2001 / Accepted: March 12, 2001

Abstract Retinoic X receptor alpha (RXRA), encoded by *RXRA*, plays a key role in development and metabolism, specifically in adipocyte biology, glucose homeostasis, and intestinal cholesterol balance. *RXRA* is also a positional candidate gene for Berardinelli-Seip congenital lipodystrophy. We report the systematic screening of *RXRA* coding regions by genomic DNA sequencing, which has resulted in the identification of three novel single-nucleotide polymorphisms.

Key words Metabolism · Genomic DNA · Sequencing · Complex traits · Obesity · Diabetes · Lipodystrophy

Introduction

The effects of retinoic acid in development and metabolism are mediated by specific nuclear receptor proteins, including the high-affinity retinoic acid receptors (RARs) alpha, beta, and gamma, and the distinct nuclear receptor called retinoid X receptor alpha, or RXRA (Mangelsdorf et al. 1990, 1991). RXRA differs from other RARs within the ligand-binding domain and can bind retinoic acid with high affinity (Heyman et al. 1992). RXRA can function both as a homodimer and as a heterodimer with other RARs, and plays a focal role in adipocyte metabolism (Willy et al. 1995). In addition, the nuclear receptor peroxisome proliferator-activated receptor gamma (PPARG)/RXRA heterodimer regulates glucose and lipid homeostasis (Gampe et al. 2000). Furthermore, RXRA has been shown to have a crucial role in cholesterol balance, intestinal cholesterol absorption, and bile acid synthesis (Repa et al. 2000). Finally, *RXRA*, which encodes RXRA, has been mapped to chromosome 9q34 (Jones et al. 1993) within the minimal linkage region for Berardinelli-Seip congenital lipodystrophy (OMIM 269700), and is considered to be a good candidate gene for this disease (Garg et al. 1999). Thus, developing genomic amplification primers for, and finding genomic variation in *RXRA* are important. We report genomic DNA amplification primers for most of the coding region of *RXRA*, and characterize three common single-nucleotide polymorphisms (SNPs), which can serve as markers for genetic linkage or association studies.

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Methods

Study subjects

Genomic DNA from 2 subjects with typical Berardinelli-Seip congenital lipodystrophy by clinical and biochemical criteria, and genomic DNA from 8 subjects with partial lipodystrophy but normal *LMNA* gene sequence (Cao and Hegele 2000) was screened. In addition, samples from a DNA archive of 30 clinically normal Caucasian subjects, 20 normal African subjects, 20 normal Chinese subjects, and 20 normal East Indian subjects were screened to determine SNP allele frequencies.

Screening *RXRA* for DNA variants

In order to amplify coding regions and intron-exon boundaries from genomic DNA, a primer set was developed, using GeneBank sequences (accession numbers NM002957 and AQ917566 through AQ917583). Primer sequences for *RXRA* exons 2 through 10 are shown in Table 1. There were insufficient reference data to design primers for exon 1. Primers were each designed to anneal at a single temperature, which allowed for the use of a single amplification apparatus. Amplification conditions were: 94°C for 5 min, followed by 30 cycles, comprised of 30 s each, of denaturing at 94°C, annealing at 58°C, and extension at 72°C, then a final extension step at 72°C for 10 min.

Table 1. Amplification primers for *RXRA*

Exon	Primer sequence	Product size (bp)
2	F: 5′-AGT CAC CTG CAC TGA CCA CTC T-3′ R: 5′-GTC CTG CAG TTG AAG CCA C-3′	364
3	F: 5′-GGG GAC ATA GGG ACA AAC CT-3′ R: 5′-CCA CAG GAG GGT GAT TTG G-3′	300
4	F: 5′-GTA GTG GCG GCG TTG GAT-3′ R: 5′-ACT CAC TCC CTG TTG TCC ATC T-3′	360
5	F: 5′-ACA GCC TTC CCT GGG AGC-3′ R: 5′-GGT GAG TGA GTG AGG GAA ACT G-3′	300
6	F: 5′-TGT TTG GTC AGT ACA CTG GCT T-3′ R: 5′-GGG AGT GTC CTG GTA CGT GT-3′	256
7	F: 5′-AGA CAG CTG AGT GAC TGT GTG C-3′ R: 5′-GAA ATA ATA CTA GGC AGG ATG TGC-3′	269
8	F: 5′-CCT TGG GTA TCT GGG GTG T-3′ R: 5′-GGC AAG AAG TAC AGC CAG TAG G-3′	255
9	F: 5′-CTG AGG GTT CTG ACC TGT GG-3′ R: 5′-GTG CCA CCT TCT CAT TCA CAT A-3′	240
10	F: 5′-CAG TGC CAG GGC AGA ACT-3′ R: 5′-CTG AGA AGA ACA GCT GGC GT-3′	279

Table 2. *RXRA* SNP allele frequencies

SNP name	Ethnic group (number)	Allele frequency	Detection method
Intron 7 +69A/G	Caucasian ($n = 30$)	+69A: 0.28	*Fnu*4HI digestion
	African ($n = 20$)	+69A: 0.05	
	Chinese ($n = 20$)	+69A: 0.30	
	East Indian ($n = 20$)	+69A: 0.22	
Intron 9 −25G/A	Caucasian ($n = 30$)	−25G: 0.32	*Nci*I digestion
	African ($n = 20$)	−25G: 0.77	
	Chinese ($n = 20$)	−25G: 0.47	
	East Indian ($n = 20$)	−25G: 0.43	
3′-UTR 1470C/T	Caucasian ($n = 20$)	1470T: 0.05	Direct sequencing

SNP, Single-nucleotide polymorphism; UTR, untranslated region

Genotyping of SNPs

The *RXRA* intron 7 +69A/G SNP was genotyped using the primers 5′-AGA CAG CTG AGT GAC TGT GTG C-3′ and 5′-GAA ATA ATA CTA GGC AGG ATG TGC-3′. The amplification conditions were: 94°C for 5 min, followed by 30 cycles, comprised of 30 s each, at 94°C, 58°C, and 72°C, ending with a single 10-min extension step at 72°C. The resulting fragment was 269 base pairs in length. Digestion of the +69A allele with *Fnu*4HI produced three fragments, of size 162, 66, and 41 base pairs, whereas digestion of the +69G allele produced four fragments, of size 123, 66, 41, and 39 base pairs. These fragments were resolved by electrophoresis in 2% agarose gels.

The *RXRA* intron 9 −25G/A SNP was genotyped using the primers 5′-CAG TGC CAG GGC AGA ACT-3′ and 5′-CTG AGA AGA ACA GCT GGC GT-3′. The amplification conditions were: 94°C for 5 min, followed by 30 cycles, comprised of 30 s each, at 94°C, 58°C, and 72°C, ending with a single 10-min extension step at 72°C. The resulting fragment was 279 base pairs in length. Digestion of the −25A allele with *Nci*I produced a single fragment of size 279 base pairs, whereas digestion of the −25G allele produced two fragments, of size 245 and 34 base pairs. These fragments were resolved by electrophoresis in 2% agarose gels.

The *RXRA* 3′-untranslated region 1470C/T SNP did not alter a restriction endonuclease recognition site, and, thus, allele frequencies were estimated in 20 normal Caucasians, using the electrophoretogram tracings obtained from direct genomic sequencing using primers 5′-CAG TGC CAG GGC AGA ACT-3′ and 5′-CTG AGA AGA ACA GCT GGC GT-3′.

Allele frequencies were determined from electrophoretogram tracings of genomic DNA sequence, except for two SNPs that were assayed using restriction digestion. χ^2 analysis in SAS (version 6.12, SAS Institute, Cary, NC, USA) was used to test the deviation of genotype frequencies from Hardy-Weinberg predictions, with nominal $P < 0.05$.

Results and discussion

Genomic DNA sequencing experiments uncovered three relatively common SNPs in *RXRA*, designated intron 7 +69A/G, intron 9 −25G/A and 3′-untranslated region 1470C/T. No other genomic sequence mutations were detected in any study sample. The observed genotype frequencies of all SNPs did not deviate from Hardy-Weinberg predictions. The allele frequencies are shown in Table 2.

We thus report: (1) a definition of primer sets to amplify most of the coding region of *RXRA* and (2) the use of these amplification primers for genomic DNA sequencing, which has resulted in (3) the identification of three novel SNPs in *RXRA*. In addition, we have ruled out the presence of coding sequence mutations in these exons in two subjects with Berardinelli-Seip congenital lipodystrophy. The two subjects were each singleton cases from small families, and, thus, linkage analysis could not be performed to determine whether there was linkage to the region on chromosome 9q34 that harbors *RXRA* or linkage to any other genomic region. Genomic DNA from these subjects will continue to be sequenced as new candidate genes are identified and prioritized for possible involvement in this phenotype. We did not examine the *RXRA* promoter regions, and so we cannot absolutely rule out such mutations in these subjects. The *RXRA* gene product appears to be a focal component of many developmental and metabolic pathways. The reagents described in this report could be helpful to screen *RXRA* to find other markers with other diseases. The SNPs described in this report could be used in studies of association or linkage with other metabolic phenotypes.

Acknowledgments Pearl Campbell provided outstanding technical assistance. Dr. Hegele holds a Canada Research Chair in Human Genetics and a Career Investigator award from the Heart and Stroke Foundation of Ontario. This work was supported by grants from MRC Canada (MT13430), the Canadian Diabetes Association (in honor of Reta Maude Gilbert), the Canadian Genetic Diseases Network, and the Blackburn Group.

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