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Genotyping of variants rs2279238 *LXRA* and rs2695121 *LXRB* by qPCR

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Abstract

LXRA and *LXRB* encode the oxysterol-activated nuclear receptors LXR-alpha and LXR-beta, respectively, which are key regulators of lipid metabolism and neuroprotection. These receptors maintain lipid homeostasis by controlling the transcription of target genes involved in lipid metabolism, storage, transport, and cholesterol efflux - processes that are particularly relevant within the central nervous system. The protocol aims to standardize the genotyping of single nucleotide variants (SNVs) using a quantitative PCR (qPCR) approach.

Materials

- Blood samples (10 mL) with ethylenediaminetetraacetic acid (EDTA)
- Cryotubes
- PureLink® Genomic DNA Kits (ref. K-1820-02)
- Nanodrop® instrument (Thermo Fisher Scientific)
- TaqMan® hydrolysis probes
- QuantStudio 6 Pro Real-time PCR System®
- TaqMan® Genotyping Master Mix® (2X)
- Applied Biosystems™ TaqMan™ SNP Genotyping Assays® (ref. 4351379)

Before start

Be sure to wear a coat, mask and gloves. Be careful when manipulating all components of the master mix, preventing contamination.

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Materials and methods

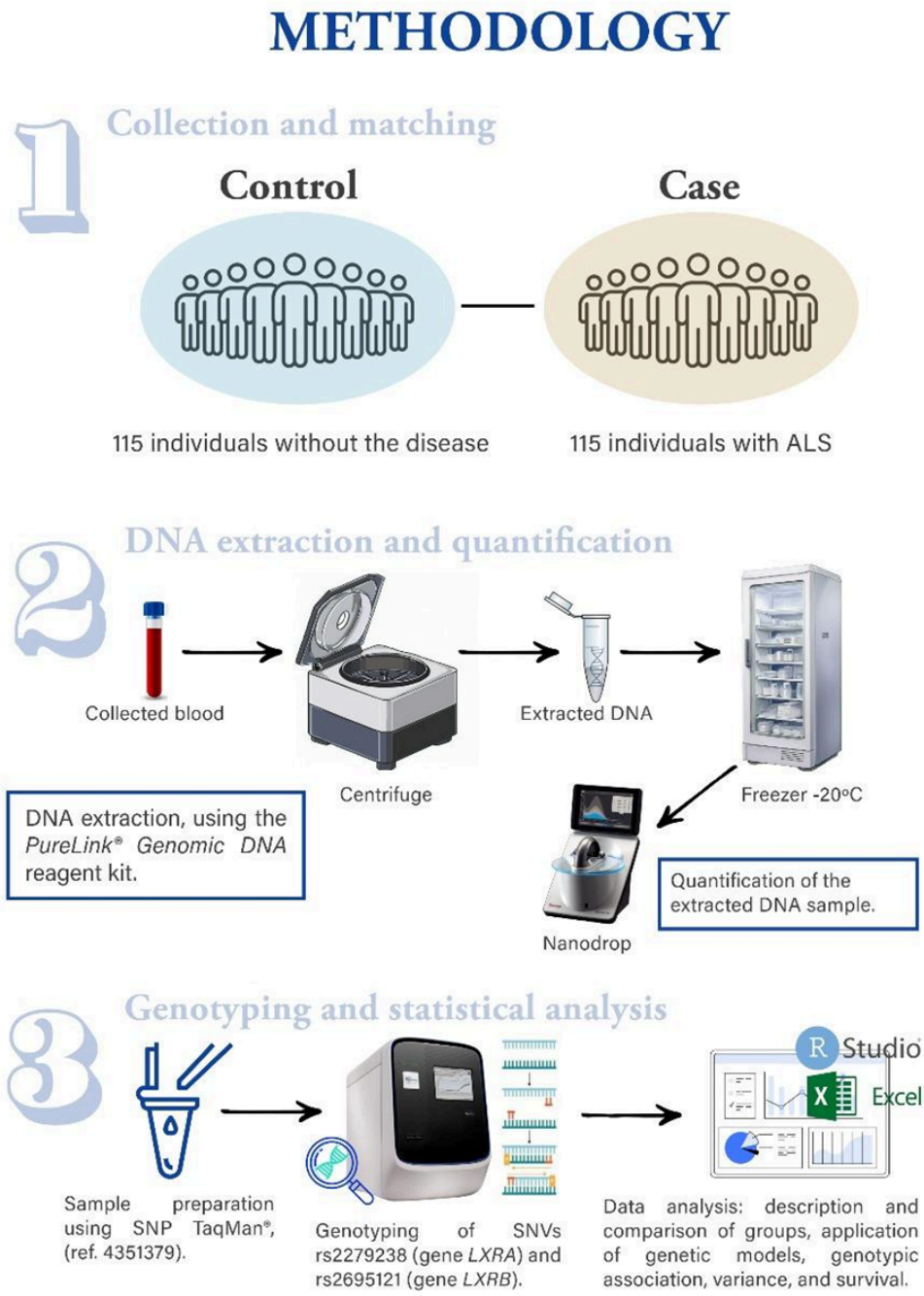
2 **Study design and ethics**

The reliability and validity of study findings depend on well-described and rigorously validated procedures that span the entire research workflow - from ethics approval and definition of the target population to site selection variable specification, materials and methods, data acquisition and analytic tools. In this protocol, genotyping of variants in the two genes under investigation was performed by the quantitative polymerase chain reaction (qPCR) technique. The study was approved by the Ethics and Research Committee of the Federal University of Goiás (CEP/UFG), Brazil, under CAAE number 79593117.7.0000.5083. Participants from both groups were invited and voluntarily agreed to participate in the research and provided written informed consent before enrollment

Variant selection

In this protocol, two SNVs were selected. The first, rs2279238 in *NR1H3* (also known as *LXRA*), is located on chromosome 11p11.2 (GRCh38), exon 4, position 47,260,473. The second, rs2695121 in *NR1H2* (*LXRB*), resides on chromosome 19q13.3 (GRCh38), in the intronic region, at position 50,377,484. The data were extracted from NCBI databases (2023) [1-2] - including dbSNP and Gene - and from relevant literature to inform the study design [3-5]. The methodological workflow is summarized in the flowchart presented in Figure 1; however, this protocol describes only the genotyping procedures.

Figure 1. Diagram illustrating the methodological workflow of genotyping.



The authors, 2026.

3 Molecular analysis

Genetic analyses were performed on blood samples (10 mL) with ethylenediaminetetraacetic acid (EDTA). Samples were stored in cryotubes at -20 ºC until processing. Genomic DNA was extracted and purified using a silica-based selective DNA-binding method in the presence of chaotropic salts (PureLink®

Genomic DNA Kits (ref. K-1820-02). DNA concentration was determined by spectrophotometry (1uL) in the Nanodrop® instrument (Thermo Fisher Scientific) [6]. TaqMan® hydrolysis probes were employed for qPCR on a QuantStudio 6 Pro Real-time PCR System® [7]. The qPCR mixture comprised: (i) Genomic DNA at 10ng/uL per reaction; (ii) TaqMan® Genotyping Master Mix® (2X); and (iii) pre-designed probes from Applied Biosystems™ TaqMan™ SNP Genotyping Assays® (ref. 4351379), as shown in Table 1.

Table 1. Target sequence corresponding to the TaqMan hydrolysis probe for *LXR* genes.

Gene	Variant	Context Sequence [VIC/FAM]	Assay ID
LXRA	rs2279238	AAATGCTGGGGAACGAGCTATGCAG**[C/T]** GTGTGTGGGGACAAGGCCTCGGGCT	C__15967384_10
LXRB	rs2695121	AGGGTTGTGGCTGAGAGTAGGGTCT**[C/T]** TGGAGAGATGGAAAAAAAAGGGAAA	C__16059177_10

Thermo Fisher Scientific®, 2017 [8].

The probe has two distinct fluorophores: VIC and FAM, responsible for fluorescence when the corresponding allele is identified in the target sequence. One allele is labeled with the fluorescent dye VIC, designed to detect allele 1 (allele C) in the corresponding target sequence, while the second allele is labeled with the dye FAM and targets allele 2 (allele T), as described in Table 2. Both variants involve a single nucleotide substitution, in which cytosine is replaced by thymine (C/T).

Table 2. Hydrolysis probes fluorescence of the alleles involving SNVs rs2279238 *LXRA* and rs2695121 *LXRB*.

Fluorescence increase	Indication	Allele
VIC dye fluorescence only	Homozygosity for allele 1	C
6FAM dye fluorescence only	Homozygosity for allele 2	T
Fluorescence signals for both dyes	Heterozygosity for allele 1-allele 2	C/T

Thermo Fisher Scientific®, 2017 [8].

The TaqMan® Genotyping Master Mix assay solution is ready to use. Its composition includes ultrapure DNA polymerase, deoxyribonucleotide triphosphates (dNTPs), ROX dye (passive reference), and buffer. To this solution, a specific hydrolysis probe from

Applied Biosystems™ TaqMan™ SNP Genotyping Assays – ThermoFisher Scientific diluted to a 20x concentration was added. The reagent and probe mixture was prepared for 106 reactions, incorporating a 10% safety margin, as recommended by the manufacturer (96 reactions + 10% margin). A volume of 9µL of the mixture and 1µL of genomic DNA diluted to 10ng/µL was dispensed into each well of the reaction plate, totaling 10µL to fill each of the 96 wells of the optical plate.

Each plate was used to genotype 92 samples from participants in the study, along with two positive controls, one heterozygous (C/T) and one homozygous mutant (T/T), and two negative controls (NTC), which contained only the reagent mixture without the presence of the DNA sample. The reagents were prepared, and the prediluted samples were distributed in a laminar flow hood, following the equipment manufacturer's protocol. After distributing the samples to all wells, the plate was coated with MicroAmp Optical Caps for 96-well plates.
The genotyping assay identifies whether a specific SNP is present or absent by monitoring variations in the fluorescence emitted by the probe-linked dyes. Table 3 outlines the quantities used for the genotyping reaction corresponding to a single assay plate.

Table 3. Description of the quantities used to perform genotyping, referring to a 96-well assay plate.

Components	1 Reaction	Units	106 Reactions	Units
TaqMan® Genotyping Master Mix-2x	5,0	µL	530	µL
TaqMan® SNP Genotyping Assays-20x	0,5	µL	53	µL
DNA-free ultrapure water	3,5	µL	371	µL
Total	9	µL	954	µL
Homogenize and distribute 9 µL on the plate				
DNA sample (10 ng/ µL)	1	µL	-	-

Thermo Fisher Scientific, 2017 [8].

The cycling protocol used followed the standard conditions provided by the manufacturer of the TaqMan® hydrolysis probes, as detailed in Table 4. In the plate software, select the Standard mode thermal cycling setting.

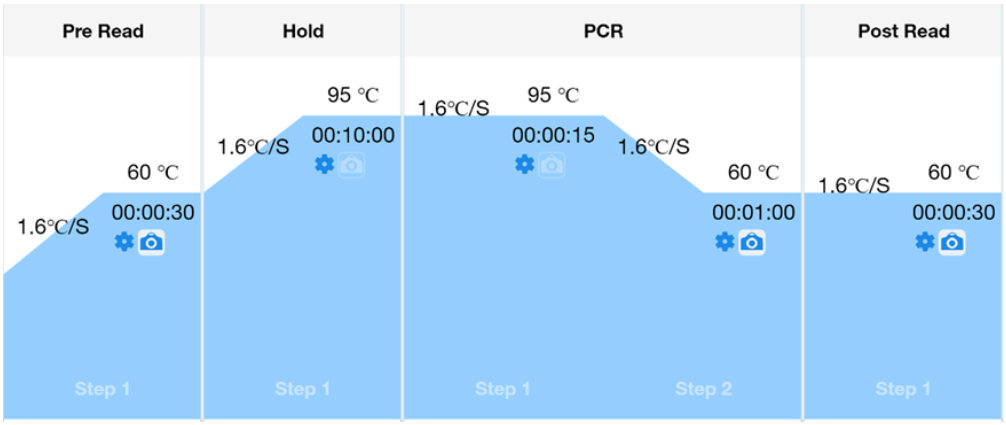
Table 4. Cycling protocol using validated genotyping assays for qPCR in QuantStudio 6 Pro.

Stage	Run Genotyping_SNP TaqMan®		
	Temperature	Duration	Cycles
Enzyme ativaction	95ºC	10 minutes	Hold
Desnaturation	95ºC	15 seconds	40
Anneal /Extend	60ºC	1 minute	

Thermo Fisher Scientific, 2017 [8].

Next, we present the standardized run genotyping workflow of analyses of *LXRs* variants, in Figure 2.

Figure 2. Standard run for genotyping *LXR* variants.



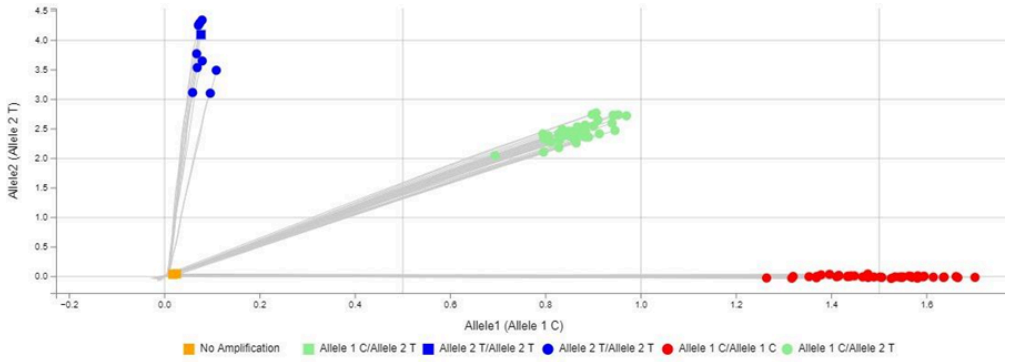
Design and Analysis software 2.7.0 da Applied biosystems – Thermofisher, 2026.

Interpretation of results

4 Genotyping results were analyzed using Diomni™ Design and Analysis (RUO) in Thermo Fisher Cloud Genotyping application, with data visualized as scatter plots. These plots display genotype clusters, each occupying a specific position and represented by a unique color. Red indicates samples with the wild-type genotype, allele 1 (typically located at the lower right quadrant of the plot. Green represents heterozygous samples, allele 1 and 2, generally positioned in the upper central region. Blue corresponds to mutant genotypes, allele 2, which appear in the upper left quadrant. Positive control samples are denoted by squares in the same color as their respective genotypes. Orange squares indicate negative controls, while orange circles represent samples that failed to amplify. This visualization methodology enhances the interpretation of genotyping results and facilitates the assessment of

data quality recorded by the software. The allelic discrimination of the rs2279238 *LXRA* gene is represented in Figure 3.

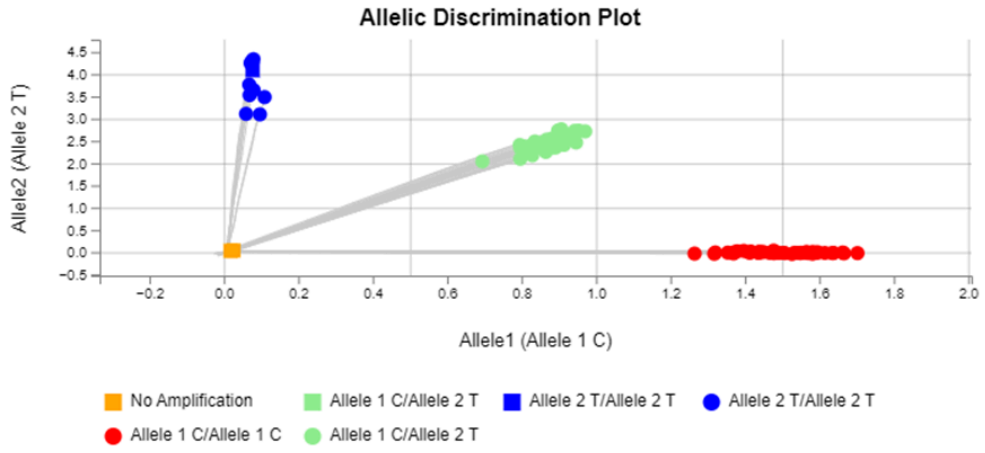
Figure 3. Allelic discrimination for genotyping of the genetic variant rs279238, *LXRA*.



Design and Analysis software 2.7.0 da Applied biosystems – Thermofisher, 2026.

The allelic discrimination of the rs2695121 *LXRB* genetic variant generated during the amplification process in QuantStudio 6 Pro is shown in Figure 4.

Figure 4. Allelic discrimination for genotyping of the genetic variant rs2695121, *LXRB*.



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