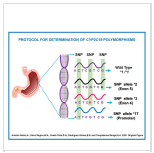

Jan 28, 2021

Prevalence of CYP2C19 Polymorphism in Bogotá, Colombia: The first report of allele *17

PLOS One

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1 Works for me dx.doi.org/10.17504/protocols.io.bn46mgze

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ABSTRACT

The aim of this protocol is to share useful information to determine the main polymorphisms of *CYP2C19*. *CYP2C19* genotyping was performed on gastric biopsy samples. Polymorphisms *1, *2, and*3 were analyzed by real-time PCR (Roche®), and PCR-RFLP was used to determine the presence of allele *17.

Protocol scope: This protocol can be used when someone want to replicate the techniques to analyze *CYP2C19* polymorphisms. In addition, the protocol provides information on how to transport, properly preserve gastric biopsies and the steps for DNA extraction from gastric biopsies.

About *CYP2C19* *2, *3 and *17 polyporphisms: they are a single nucleotide polymorphisms (SNP) in three different regions of *CYP2C19* gen that affect the characteristic of its protein and it impacts in the metabolism of some drugs such as protom pumps inhibitors (PPIs). The SNP in allele *2 occurs in exon 5 by a shif of guanine to adenine (G>A). The SNP in allele *3 occurs in exon 4 by a shif of guanine to adenine (G>A). This two SPNs reduce the ability to metabolize PPI. In the case of the SNP of allele *17 it occurs in promoter region by a shif of cytosine to thymine at -3404 and -806 positions (– 3402 C>T y - 806 C>T). The SNP in allele*17 may to recluit many transcription factors and consequently large amounts of CYP2C19 enzyme are produced, giving to the subject the ability to metabolize very quickly the PPIs. ect will be ultrarapid metabolizer. Therefore, the determination of *CYP2C19* polymorphisms are so important for precision medicine.

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0245401

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KEYWORDS

CYP2C19 Polymorphims, CYP2C19 *1, CYP2C19 *2, CYP2C19 *3, CYP2C19 *17, DNA biopses extraction, PCR-RFLP

CREATED

Oct 28, 2020

LAST MODIFIED

Jan 28, 2021

PROTOCOL INTEGER ID

43902

MATERIALS TEXT

Materials for DNA Extraction	***Materials for CYP2C19*1,*2,*3 Genotyping***	**Materials for PCR-RFLP**
QUIamp DNA mini Kit	Roche CYP2C * 19 Kit	White, Yellow and Blue Tips free of DNAse and RNAase.
White, Yellow and Blue Tips free of DNAse and RNAase.	White, Yellow and Blue Tips free of DNAse and RNAase.	DNAase and RNase-free Eppendorf tubes (2ml)
DNAase and RNase-free Eppendorf tubes (2ml)	DNAase and RNase-free Eppendorf tubes (2ml)	PCR tubes
Molecular - grade water	Glass capillaries (Roche)	Molecular - grade water
Micropipettes	Plate rack with adapters (Roche)	GoTaq Master mix (Promega)
Eppendorf Racks	Antiseptic alcohol (will be used to clean the cabin)	NsiI restriction enzyme (New England BioLabs)
Microcentrifuge	Micropipettes	Sybr Safe (Invitrogen®)
waste disposal container	PCR cabinet	Agarosa
Personal protection elements.	Absorbent napkins	water bath
Absorbent napkins	Microcentrifuge	vortex
Lab notebook	Personal protection elements.	Micropipettes
	Capin tool	Eppendorf Racks
	Light Cycler 1.5 Thermocycler	pair of the scales
	Vortex	Thermocycler
	Lab Notebook	Microcentrifuge
	waste disposal container	waste disposal container
		Personal protection elements.
		Absorbent napkins
		Lab notebook

BEFORE STARTING

1. Please read the protocols and manuscrips
2. Please wash your hands
3. Please use personal protection implements such as DNase and RNAase free gloves, face mask, hat and gown.
4. Please clean and disinfect the work site
5. Please list the necessary reagents for DNA extraction, RT-PCR, PCR-RFPL and electrophoresis.
6. Prepare the equipments and supplies such as 2ml eppendorf tubes, pipette tips, micropipette, PCR tubes, and RT-PCR caps.
7. List and identify well the samples to analyze in your laboratory notebook.
8. Mark the tubes with the name or number ID of each sample
9. When performed the tests remember to do according biosafety regulations
10. When you finish your work remember to write down the results and leave everything in order

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1 Ethics aproval

- The ethics committees from participant institutes approved the study protocol.
- Since the study included human samples It was performed in agreement with Good Clinical Practice guidelines and the ethical principles of the Declaration of Helsinki.

1.1 Participants

1. Inclusion and exclusion criteria:

- This study included subjects between 19 and 70 years old who were referred for functional dyspepsia or peptic ulcers and recommended to have endoscopy and who agreed to participate in the study.
- The protocol study excluded pregnant women; subjects with concomitant diseases such as diabetes, mental disorders, gastric atrophy or intestinal metaplasia; subjects with previous gastric cancer; and subjects with previous gastric surgery.

2. Subjects recruitment:

- The subjects were enrolled randomly in the study during gastroenterology consultation after a gastroenterologist explained to them that the information derived from this research could help to select in a better way the PPIs that are used for patients with peptic acid diseases.
- Written informed consent was obtained from subjects who met the inclusion and exclusion criteria by the endoscopic service.

1.2 Samples

1. Samples obtention:

- The sample used was gastric biopsy.
- Endoscopy was performed by an expert gastroenterologist with an Exera Olympus CV 145 video endoscope. Endoscopy was performed after six hours of fasting with the standard methodology and with sedation depending on the tolerance of the procedure (on demand).

Park KS (2015). Introduction to Starting Upper Gastrointestinal Endoscopy: Proper Insertion, Complete Observation, and Appropriate Photographing.. Clinical endoscopy. https://doi.org/10.5946/ce.2015.48.4.279

- During the procedure, biopsies of the upper digestive tract were obtained according to established protocols independent of visible pathologies, and a gastric body biopsy sample was taken for the molecular analysis of CYP2C19 genetic polymorphisms.

Technology Assessment Committe., Barkun A, Liu J, Carpenter S, Chotiprasidhi P, Chuttani R, Ginsberg G, Hussain N, Silverman W, Taitelbaum G, Petersen BT (2006). Update on endoscopic tissue sampling devices.. Gastrointestinal endoscopy.

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Allen JI, Katzka D, Robert M, Leontiadis GI (2015). American Gastroenterological Association Institute Technical Review on the Role of Upper Gastrointestinal Biopsy to Evaluate Dyspepsia in the Adult Patient in the Absence of Visible Mucosal Lesions.. Gastroenterology.
https://doi.org/10.1053/j.gastro.2015.07.040

2. Transport and store of samples for molecular analysis:

The biopsy samples was transported in 500 µL of Brucella broth (Becton Dickenson®) plus 20% (v/v) glycerol (Invitrogen®) and was kept refrigerated until it was processed.

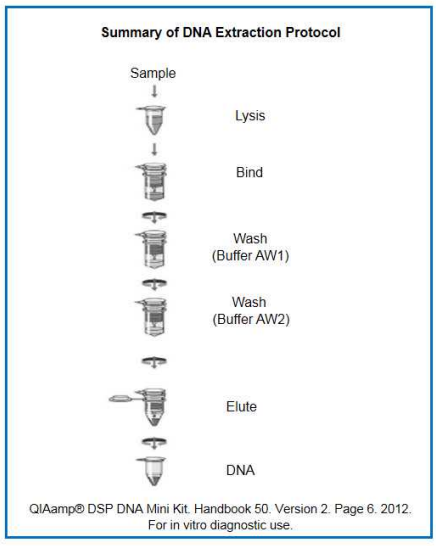
2 Statistical Analysis

Characteristics of the population and *CYP2C*19* genetic polymorphism frequencies were analyzed using descriptive statistics, employing the SPSS v.24 statistics program. Bioinformatic analysis of sequences for allele *CYP2C19*17* was performed with the BLAST-N tool Nucleotide BLAST. The alignments were performed with the wild-type reference sequences (GenBank Access: AL583836 and NG_008384.3.18)

2.1 DNA Extration

3m 30s

The QIAamp DNA Mini kit **Contributed by users** was used to obtain DNA from gastric biopsy samples according to the manufacturer's instructions **Protocol QIAamp DNA Mini Kit.pdf** with small variation in steps 1, 2, 9 and 10 as follow:



1. Take a gastric biopsy (antrum and /or body stomach tissue) of each patient, and resuspend in eppendorf with **80 µl of PBS 1X**
2. With the activated carbon swab, macerate the biopsy completely and again add **80 µl of PBS 1X**

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3. Add **100 µl of Buffer ALT** and incubate at **56 °C with shaking overnight** . Centrifugate **1500 rpm, 00:01:00 , to remove the drops fron the lid**

4. Add **200 µl Buffer AL** . Mix thoroughly by vortexing for **00:00:15 and incubate at 70 °C for 10 minutes** and briefly centrifuge the tube to remove drops from the lid.

5. Add **200 µl ethanol 96-100%)** . Vortex for **00:00:15** . Briefly centrifuge the tube to remove drops from the lid.

6. Pipet the mixture onto the QIAamp Mini spin column (in a **2 mL collection tube**). Centrifuge at **6000 x g, 00:01:00** or **8000 rpm, 00:01:00 , and discard the fow-through and collection tube**

7. Place the QIAamp Mini spin column in a new **2 mL collection tube and add 500 µl of buffer AW1** Centrifuge at **6000 x g, 00:01:00** or **8000 rpm, 00:01:00** . Discard the flow-through and collection tube.

8. Place the QIAamp Mini spin column in a new **2 mL collection tube and add 500 µl Buffer AW2.** Centrifuge at full speed (**20.000 x g, 00:03:00** or **14000 rpm, 00:03:00**) Discard the flow-through and collection tube.

9. **Recommended:** Place the QIAamp Mini spin column in a new **2 mL collection tube (not provided) and centrifugate at full speed for 00:01:00** min. This eliminates the chance of possible Buffer AW2 carryover.

10. Place the QIAamp Mini spin column in a new **1.5 mL microcentrifuge tube (not provided), add 200 µl warm molecular grade water** and incubate at **Room temperature 00:01:00** . Centrifuge at **6000 x g** or **8000 rpm, 00:01:00 , for 00:01:00 to elute the DNA.**

10. **Optional:** Repeat step 9 for increased DNA yield with a further **200 µl warm molecular grade water** .

3 Molecular Analisys

3.1 CYP2C19 *1, *2, *3 Genotyping

CYP2C19 *1, *2, and *3 genetic polymorphisms were determined by real-time polymerase chain reaction (RT-PCR) using the LightMix® kit for human CYP2C19*2 and CYP2C19*3 (Roche®). PCR was performed according to the manufacturer's instructions. CYP2C19 *2 and CYP2C19 *3 Experimental Protocol Roche **Protocol LightMix CYP2C19 2 and 3.pdf**

1. Each PCR was carried out in a final volume of **20 µl as follows: 9.4 µl of molecuar-grade water (Tib Molbiol), 1.6 µl** $MgCl_2$, **2 µl primers, 2 µl probes, 2 µl Master mix and 5 µl of DNA.**

2. PCR was performed in a LightCycler 1.5. Before initial analysis, color compensation in reading channels was performed to guarantee good results, and quality control for each allele (wild type and mutant alleles 2 and 3) was included for every test.

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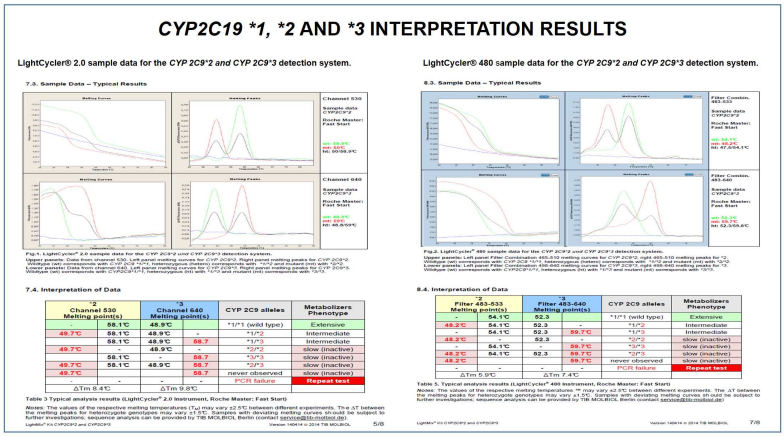
3. Allelic classification was analyzed by differences in melting temperatures (Tm) (curves obtained) in channel 530 for allele 2, with Tm between 48.6°C and 54.4°C, and in channel 640 for allele 3, with Tm between 53.4°C and 60.8°C, according to the manufacturer's suggestion as follow:



3.2 CYP2C19 *17 Genotyping

8h

PCR and RFLP were used to determine the *CYP2C19 *17* genetic polymorphism. This PCR was standardized according to previous reports by Baldwin et al. 2008

> Baldwin RM, Ohlsson S, Pedersen RS, Mwinyi J, Ingelman-Sundberg M, Eliasson E, Bertilsson L (2008). Increased omeprazole metabolism in carriers of the CYP2C19*17 allele; a pharmacokinetic study in healthy volunteers.. British journal of clinical pharmacology.
> https://doi.org/10.1111/j.1365-2125.2008.03104.x

This nested PCR and RFLP consisted of two PCRs and a final enzymatic digestion from the product of the second PCR.

1. First, PCR was carried out in a final volume of **10 µl as follows:** as follows:
3.2 µl of molecular grade water (Sigma®), **0.4 µl primers** (10 µM),

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5 µl Master mix

GoTaq(R) Green Master Mix, 100 Reactions **Promega Catalog #M7122** and

1 µl DNA.

2. The primer pair used in this first PCR and the cycling conditions are showing in table 1. Primers were synthesized by Invitrogen USA and amplified a 473 bp fragment, corresponding to *CYP2C19* allele 1.

CYP2C19 *17 **PCR AND RFLP AMPLIFICATION PROTOCOL**

(Table 1)

Author	Primers		Initial Denaturation	Denaturation	Annealing	Extension	Final Extension	Product (pb)	Product (pb) post- NsiI
Baldwin, *et al*, 2008	2C19-1 F	5-GCCCTTAGCACCAAATTCTC-3	95°C x 1min	95°C x 30s	52°C x 30s	72°C x 30s	72°C x 7min	473	116
	2C19-1 R	5-ATTTAACCCCCTAAAAAAACACG-3		x 35 Cycles					
	2C19-2 F	5-AAATTTGTGTCTTCTGTTCTCAATG-3	95°C x 1min	95°C x 30s	51°C x 30s	72°C x 30s	72°C x 7min		143
	2C19-2R	5-AGACCCTGGGAGAACAGGAC-3		x 25 Cycles					

3. The second PCR was performed using **0.5 µl of the first PCR product.** It was carried out in a final volume of **30 µl as follows:** as follows:

13 µl of molecuar-grade water (Sigma), **0.75 µl primers** (10 µM),

15 µl Masater mix

GoTaq(R) Green Master Mix, 100 Reactions **Promega Catalog #M7122** and

0.5 µl of DNA of DNA and using another set of primers synthesized by Invitrogen USA listed in table 1 with the PCR cycling conditions.

4. Next, **15 µl of the second PCR reaction was incubated with**

0.8 µl of NsiI restriction enzyme at **37 °C for** **08:00:00 .**

5. Subsequently, the PCR digestion productwas revealed on

2 Mass / % volume agarosa gel and stained with

SyberSafe DNA Gel Stain **Invitrogen - Thermo Fisher Catalog #S33101** to verify the presence of the 116 bp and 143 bp bands, corresponding to the *CYP2C19*1* and *CYP2C19*17* alleles, respectively (Figure: *CYP2C19*17* PCR-RFLP Gel Eectrophoresis Results) .

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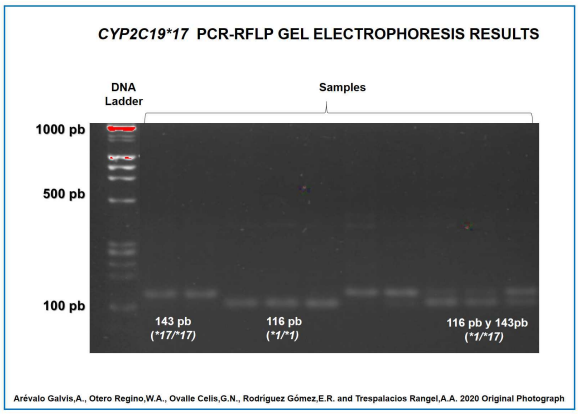

6. In addition, to confirm the presence of *CYP2C19*17* by nested PCR and RFLP, 18.4% of samples were selected randomly and sequenced (Macrogen, Korea).

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