



# CHARACTERIZATION OF CANNABINOID 1 RECEPTOR ISOFORMS IN FETAL AND MATERNAL TISSUES

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## INTRODUCTION

Along with alcohol and tobacco, marijuana is the most commonly used substance among women in childbearing age. Evidence shows that marijuana during pregnancy influences fetal development. However, specific effects of marijuana on pregnancy remain unclear. Endogenous cannabinoid system (ECS) is composed of cannabinoid receptors (CB1R and CB2R), cannabinoids (AEA and 2-AG), and synthesizing/degrading enzymes.

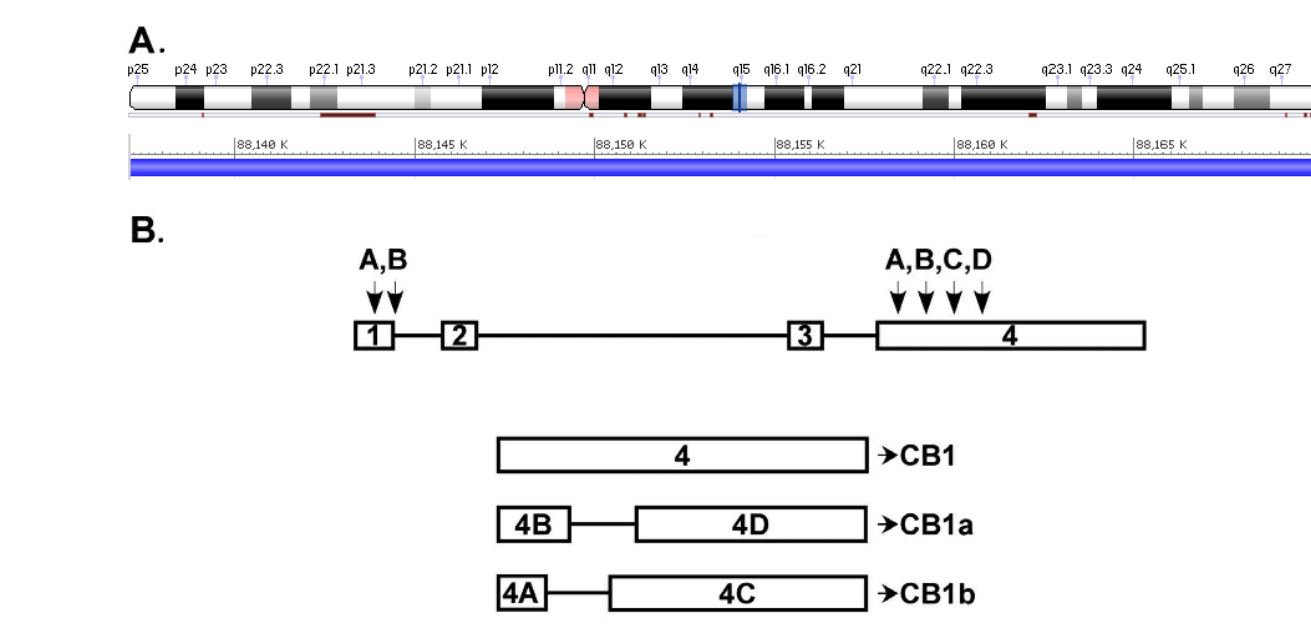
Human cannabinoid receptor 1 (*CNR1*) gene encodes unique CB1R protein isoforms. Translation of the complete *CNR1* coding exon produces a full length CB1R protein isoform while an intra-exonal splicing at the N-terminus coding region a shorter sequence for CB1<sub>a</sub>R isoform and an in-frame deletion for CB1<sub>b</sub>R protein isoform. CB1R isoforms differ in their ligand binding site and signaling responses, and have an exclusive pharmacological profile for the treatment of metabolic-related conditions.

## OBJECTIVE

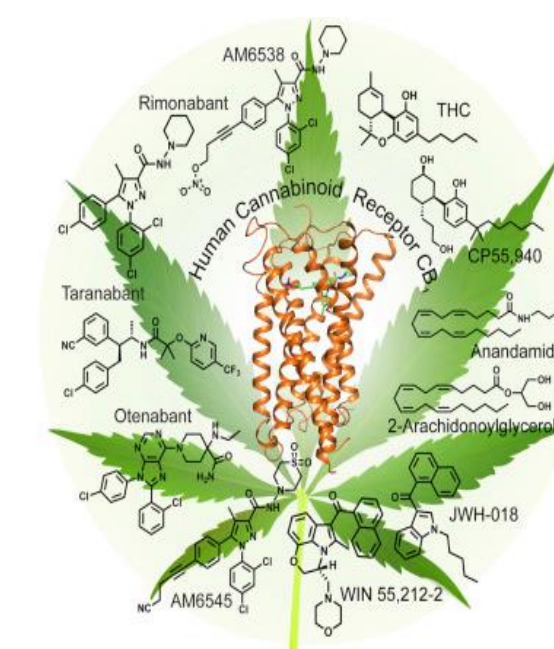
To characterize the mRNA and protein expression of CB1R full length, CB1<sub>a</sub>R, and CB1<sub>b</sub>R isoforms isolated from fetal and maternal tissues.

## MATERIALS AND METHODS

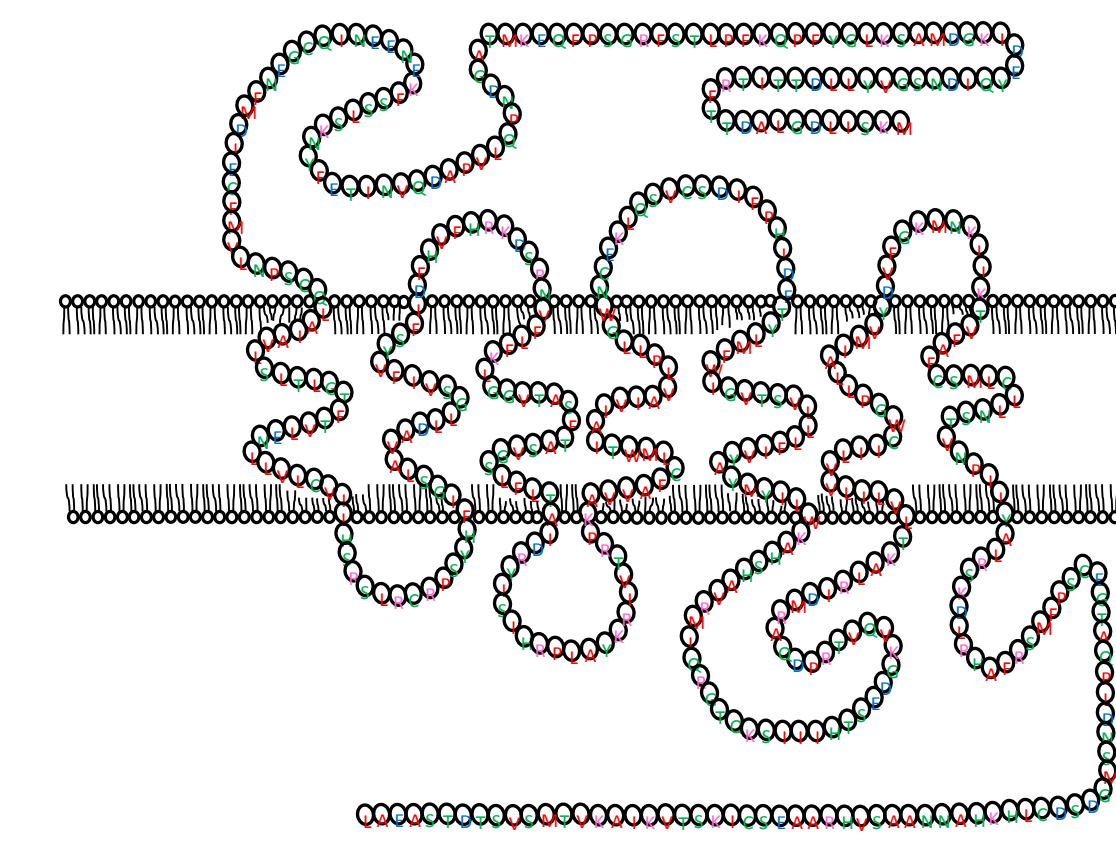
Maternal and fetal tissues were available from the tissue bank at the Southwest National Primate Research Center, Texas Biomedical Research Institute (San Antonio, TX, USA). Protein expression of CB1R isoforms was assessed by Western blot analyses and quantified by using a ChemiDoc-It<sup>TS3</sup> Imager and Image J software. Anti-CB1 (Mouse) Monoclonal Primary Antibody (Cat. #: IMG-CB1R-mAb001, Immunogenes, USA) (5% BSA in TBS-T /overnight) and peroxidase Donkey Anti-Mouse secondary antibody (Cat. #: 715-035-150, Jackson ImmunoResearch Laboratories, Inc. USA) at 1:10000 in 5% BSA in TBS-T (1X) were used. Total RNA was extracted from the tissue samples using TRIzol reagent according to the manufacturer's instructions and SYBR Green mix (Kapa Bio systems Inc. Woburn, MA, USA) used to perform Q-RT-PCR assays in a LightCycler® 96 (Applied Biosystems/Roche, USA). A standard  $\Delta\Delta Ct$  method was used to present the relative mRNA expression in contrast to  $\beta$ -actin as reference gene.



**Figure 1.** *CNR1* [*Homo sapiens* (human)] chromosomal location and structure diagram. A. Location: 6q15 (NCBI). B. Bars with numbers inside represent exons and horizontal lines represent introns. [Modified from Gonzalez-Mariscal et al., 2016].

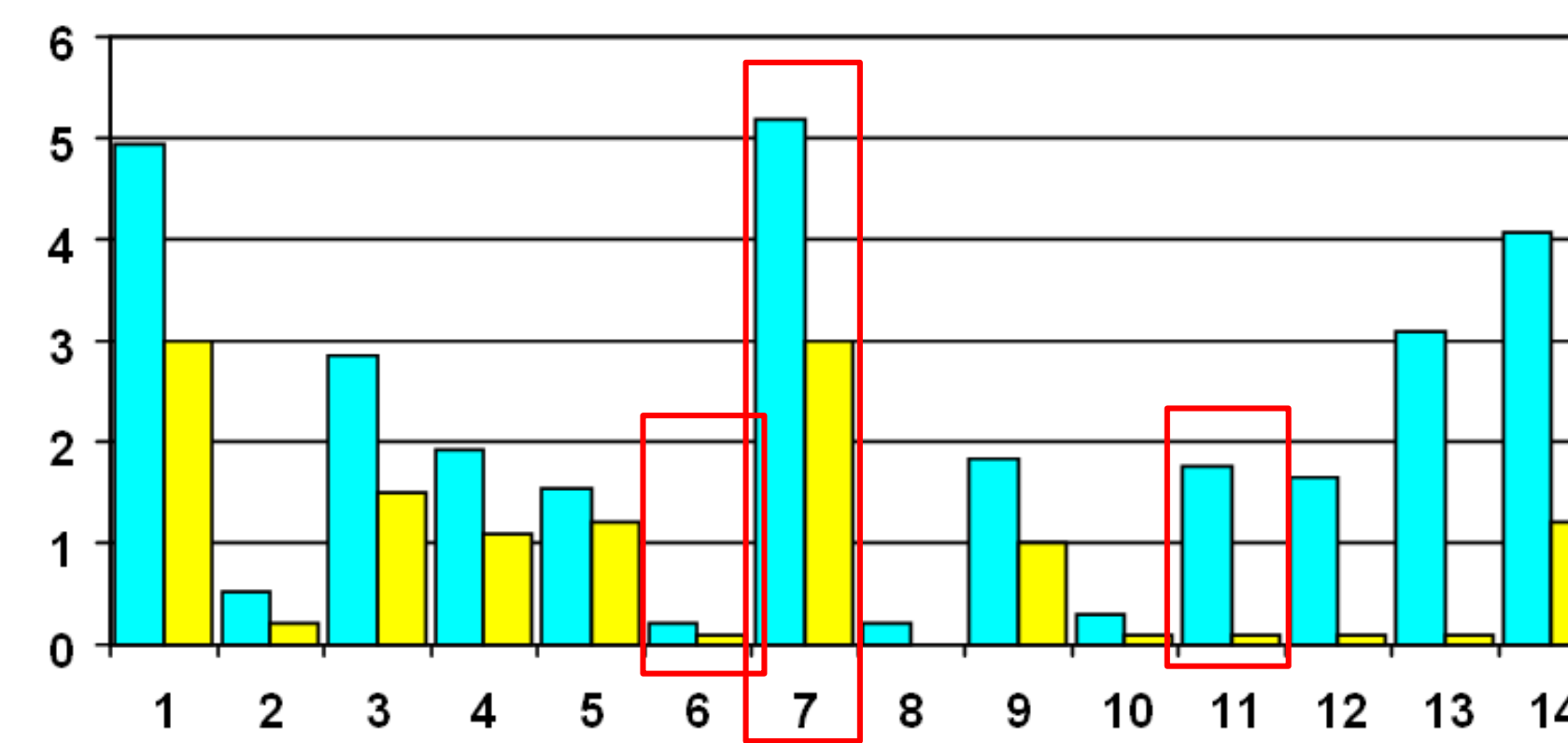


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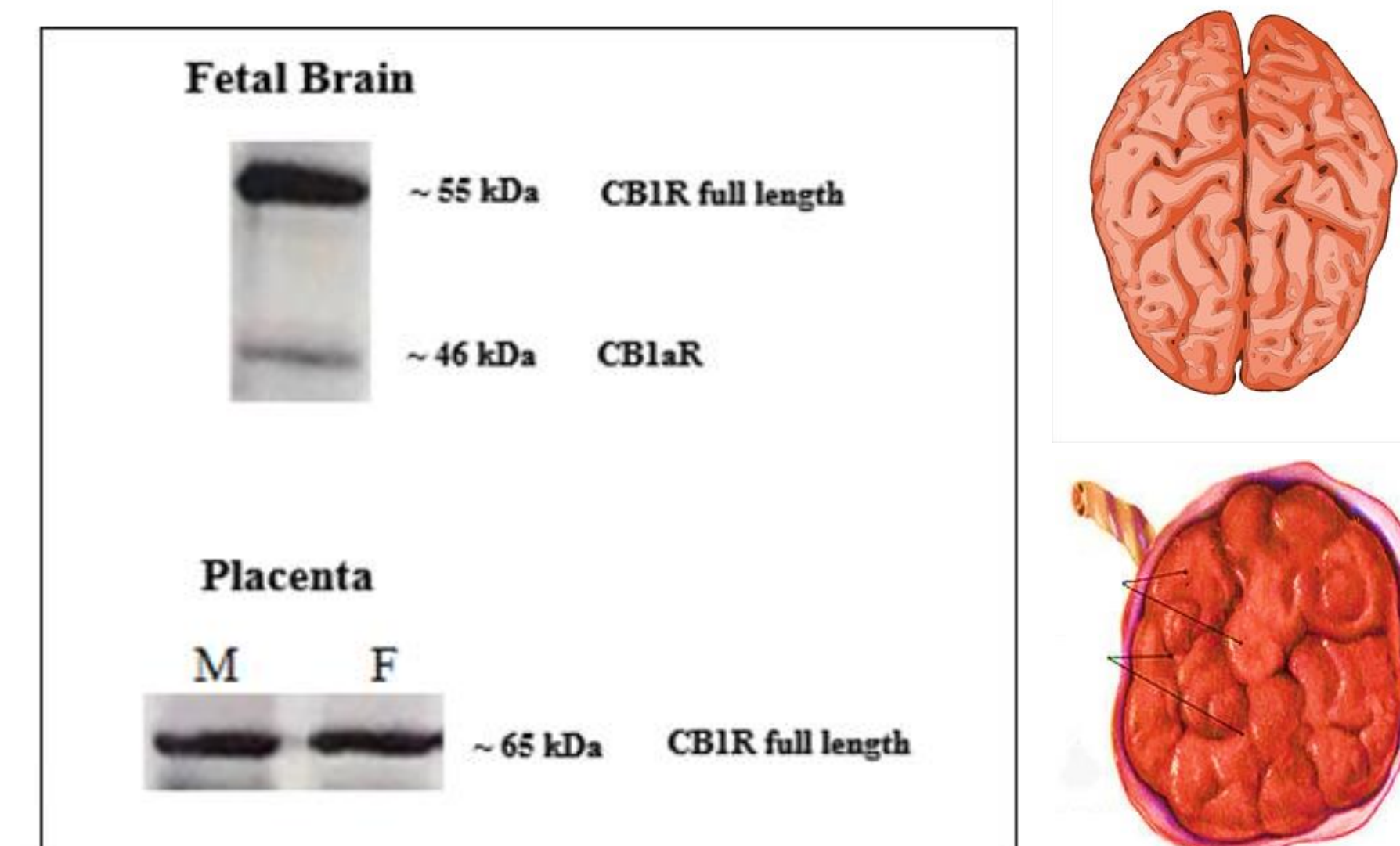
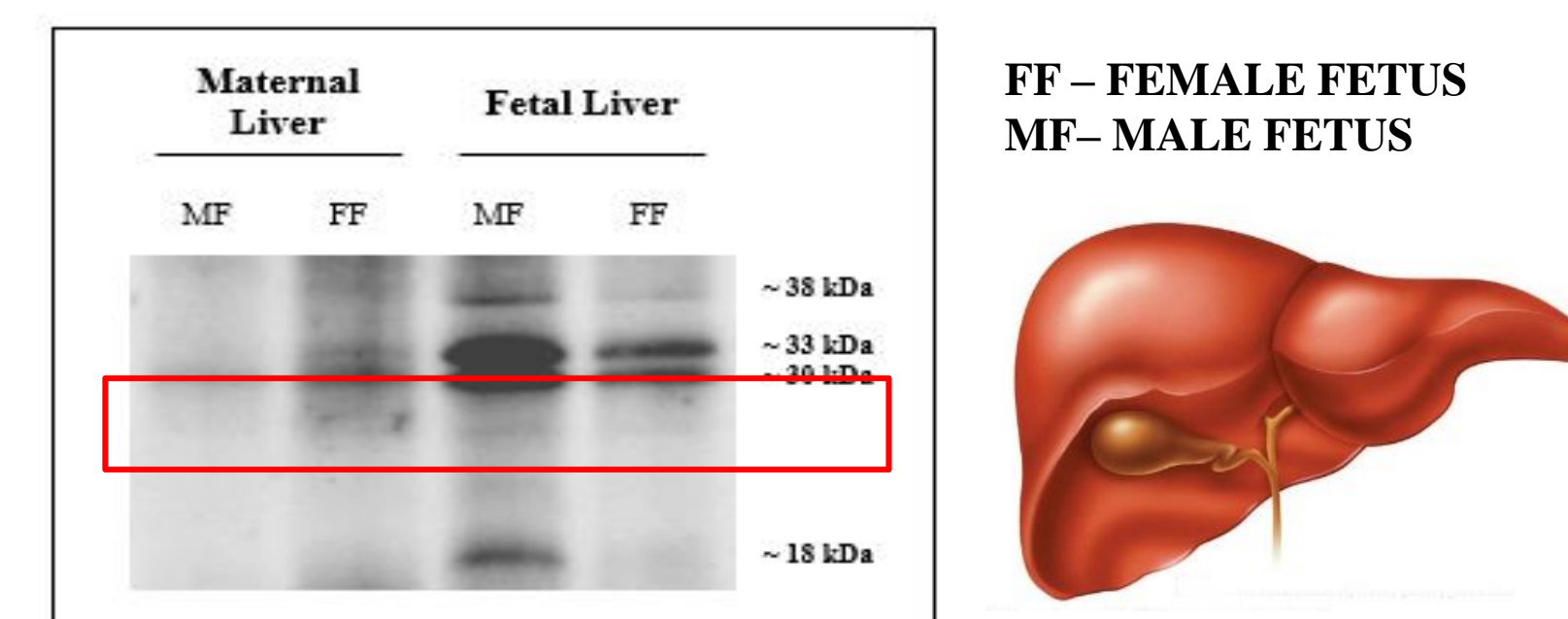
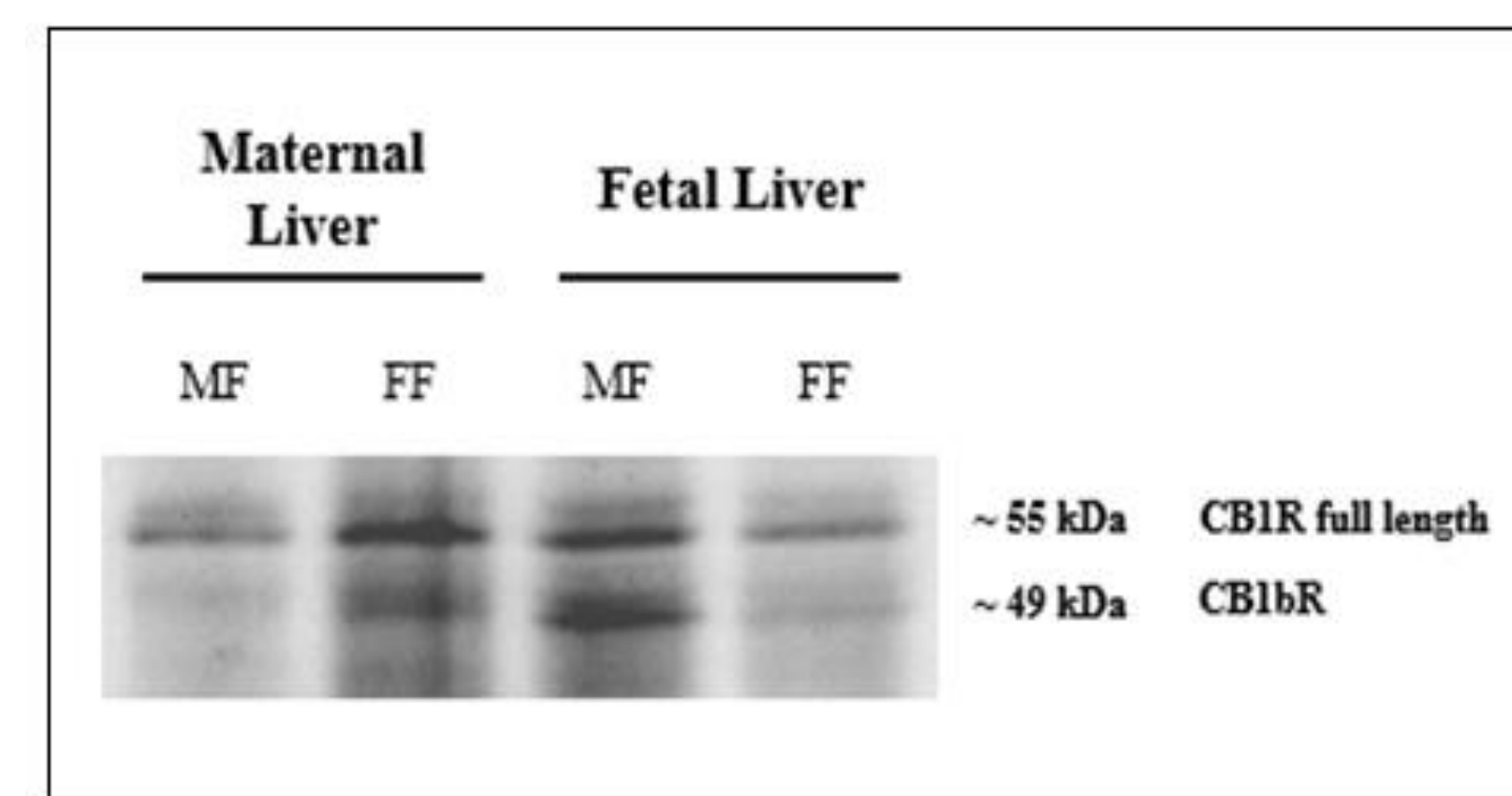


**Figure 2.** Protein structure of CB1R full length. [Dr. Guindon, Poster at AMERSA meeting, 2016].

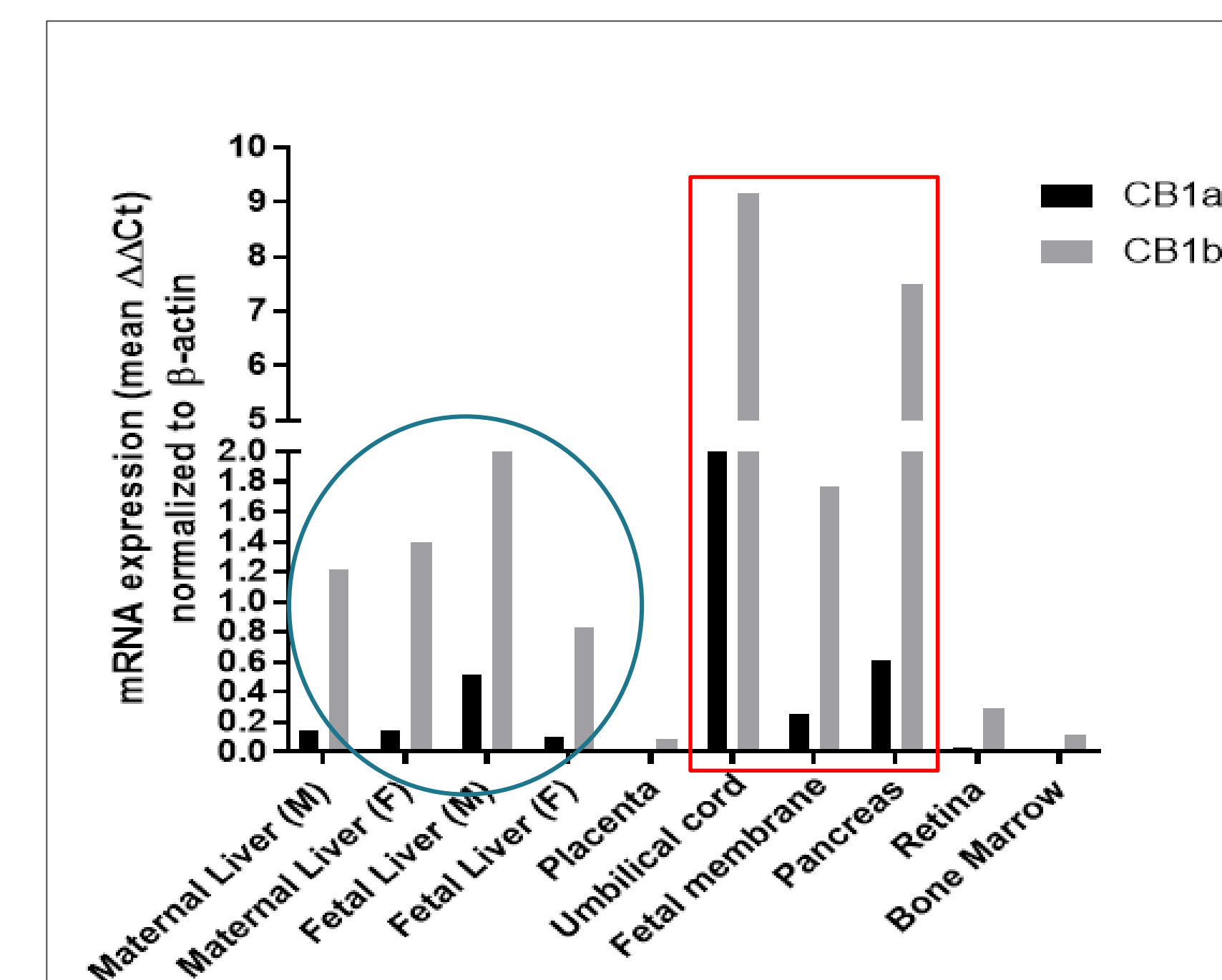
## RESULTS



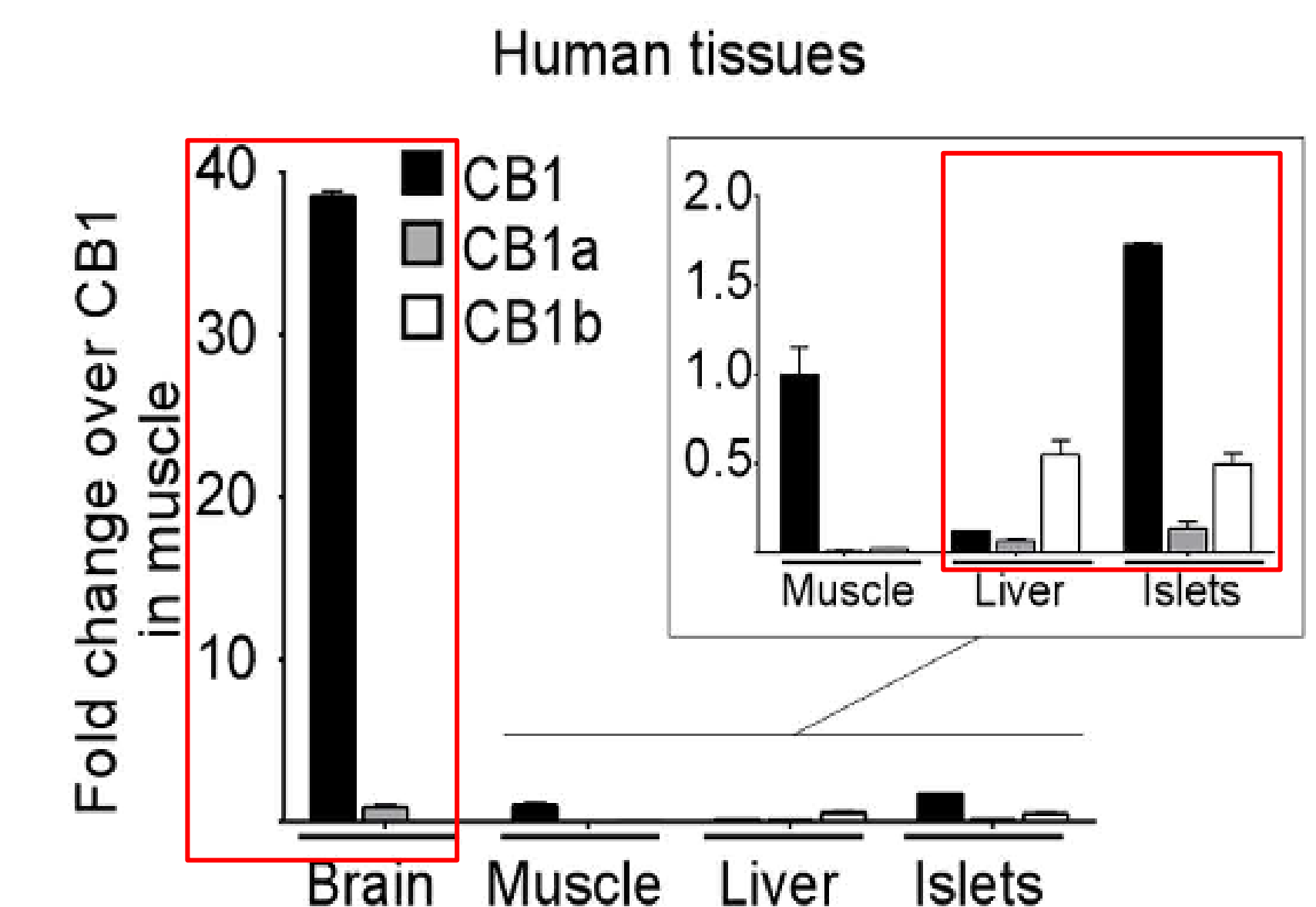
**Figure 3.** CB1 (blue) and CB2 (yellow) cDNA tissue distributions and relative abundance analyses. 1.-stomach, 2.-hypothalamus, 3.-liver, 4.-ovary, 5.-kidney, 6.-Placenta, 7.mononuclear cells, 8.-colon, 9.-omental fat, 10.-subcutaneous fat, 11.-pancreas, 12.-muscle, 13.-small intestine and 14.-lung. Source: Dr. I. Rodriguez-Sanchez.



**Figure 4.** Protein expression for CB1R isoforms (kDa) in liver, brain, and placental tissues of baboon (*Papio* spp.).



**Figure 5.** Quantification of relative mRNA expression using  $\Delta\Delta Ct$  method in baboon (*Papio* spp) tissues.



**Figure 6.** Real time PCR quantification of relative expression of CB1 receptor isoforms in brain and peripheral tissues Source: [González-Mariscal et al., 2016]

## DISCUSSION

- CB1bR isoform expression is higher in umbilical cord and pancreatic tissues compared to other tissues.
- CB1R full length protein expression is similar in brain and placenta, but CB1aR isoform is absent in placenta.
- Novel short CB1R isoforms are discovered in the fetal liver.
- CB1R expression is decreased in female fetal liver compared to male fetal liver.

## CONCLUSION

1. Pharmacological agents targeting CB1R, including marijuana and cannabis derivatives might have pregnancy-specific effects:
  - Hepatic responses, specific for fetuses.
  - Potential susceptibility of male fetuses to the intervention.
2. Similarity between umbilical cord (UC) and pancreas on CB1R expression might indicate metabolic similarity between the two tissues and it is in line with reports of the ability of UC cells to produce insulin.

## REFERENCES

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- González-Mariscal, I., Krzysik-Walker, S., Doyle, M., Liu, Q.R., Cimbro, R., Calvo, S., Ghosh, S., Cieśla, Ł., Moaddel, R., Carlson, O., Witek, R., O'Connell, J., & Egan, J. (2016). Human CB1 receptor isoforms, present in hepatocytes and  $\beta$ -cells, are involved in regulating metabolism. *Scientific Reports*, 6 (33302), 1-12.

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