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# Analysis of the transcriptional regulation of the *FABP2* promoter haplotypes by PPARγ/RXRα and Oct-1

Mike Böhme, Inke Nitz, Frank Döring, Maja Klapper *

*Molecular Nutrition, Institute of Human Nutrition and Food Science, Christian-Albrechts-University of Kiel, Heinrich-Hecht-Platz 10, D-24118 Kiel, Germany*

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ABSTRACT

Variants of the human intestinal fatty acid binding protein 2 gene (FABP2) are associated with traits of the metabolic syndrome. Relevant FABP2 promoter polymorphisms c.-80_-79insT, c.-136_-132delAGTAG, c.-168_-166delAAGinsT, c.-260G>A, c.-471G>A, and c.-778G>T result in two haplotypes A and B. Activation of haplotypes by rosiglitazone stimulated PPARγ/RXRα leads to 2-fold higher activity of haplotype B than A. As shown by chimeric FABP2 promoter constructs, the higher responsiveness of FABP2 haplotype B is mainly but not solely determined by polymorphism c.-471G>A. As shown by EMSA and promoter–reporter assays, Oct-1 interacts with the −471 region of FABP2 promoters, induces the activities of both FABP2 promoter haplotypes and abolishes the different activities of haplotypes induced by rosiglitazone activated PPARγ/RXRα. In conclusion, our findings suggest a functional role of PPARγ/RXRα and Oct-1 in the regulation of the FABP2 gene.


## 1. Introduction

The human fatty acid binding protein 2 gene (FABP2, GenBank: NM_000134.2) is located on chromosome 4q28-q31, contains four exons and codes for a 15.1-kDa protein comprising 132 amino acid residues [1]. It is expressed in the intestine with highest cellular concentrations in the jejunum [2,3]. FABP2 is involved in intestinal fat absorption [4–6]. A huge number of studies in different human populations revealed associations between the minor allele of the FABP2 Ala54Thr polymorphism and risk parameters of insulin resistance [7]. Functional studies showed increased binding affinity of the variant protein to long-chain fatty acids [8]. More recently [9–11], single nucleotide polymorphisms and insertion/deletion sites c.-80_-79insT, c.-136_-132delAGTAG, c.-168_-166delAAGinsT and SNPs -260G>A, c.-471G>A and c.-778G>T were identified in the 5¢ upstream promoter region of FABP2. These polymorphisms are in complete linkage disequilibrium resulting in only two haplotypes A>B. FABP2 promoter haplotypes were associated with postprandial triglyceride levels [12], BMI [9] and type 2 diabetes [11]. In accordance with the association studies, male FABP2 knock-out mice showed key symptoms of the metabolic syndrome [13,14]. Therefore, the expression level of FABP2 seems to be critical for insulin sensitivity and triglyceride metabolism. Thus, the transcriptional regulation of the FABP2 gene is of special interest. In the present study we focus on the regulation of FABP2 promoter haplotypes by PPARγ/RXRα and Oct-1.

## 2. Materials and methods

### 2.1. Cell culture

HeLa and CaCo2 cells were purchased from German National Resource Centre for Biological Material (DSMZ, Braunschweig, Germany). Cells were maintained in MEM (Invitrogen, Carlsbad, CA, USA), supplemented with 10% (HeLa) or 20% (CaCo2) fetal calf serum (Invitrogen) and 1 mM non-essential amino acids (PAA, Cölbe, Germany) in a humidified incubator at 37 °C under an atmosphere of 5% $CO_2$. Cells were passaged at pre-confluent densities by use of 0.05% trypsin/5 mM EDTA (Biochrom AG, Berlin, Germany) solution every 2–3 days.

### 2.2. FABP2 promoter luciferase constructs

The dual luciferase system was used (Promega, Madison, WI, USA). Cloning procedures were performed using Gateway Technology (Invitrogen), described previously [11,15].

### 2.3. Chimera constructs

Mutations in haplotype A (pGL4.10[luc2]–FABP2(A)) and haplotype B (pGL4.10[luc2]–FABP2(B)) containing promoter constructs were introduced to generate chimeras with single polymorphisms of haplotype B in the background of haplotype A (A-xB) and vice versa (B-xA). Single polymorphism exchange chimera constructs are shortly named as follows: for exchange of polymorphism c.-80_-79insT of haplotype A (rs5861422): A-80B; for c.-136_-132delAGTAG

* Corresponding author. Tel.: +049 431 8805661; fax: +049 431 8805658.
*E-mail address:* klapper@molnut.uni-kiel.de (M. Klapper).

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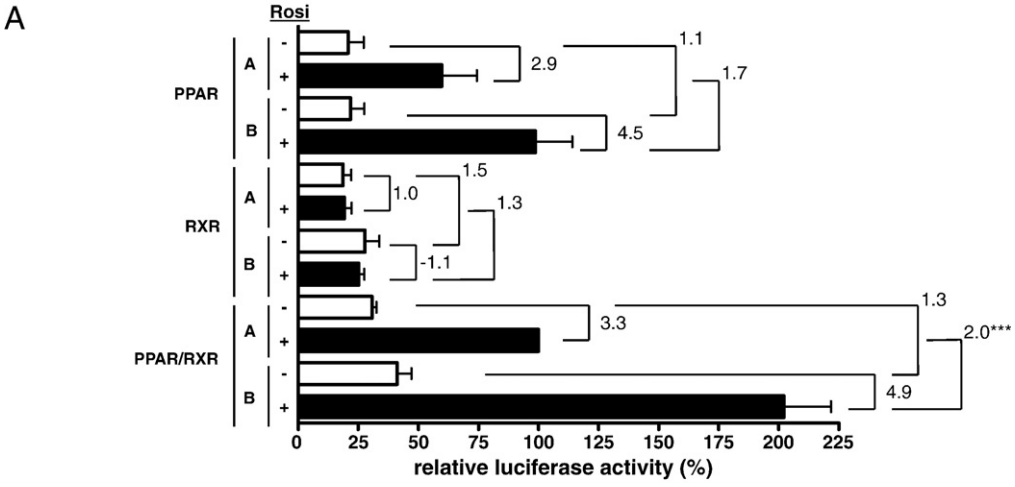
(rs5861423): A-136B; for c.-168_-166delAAGinsT (rs1973598): A-168B, c.-260G>A (rs6857641): A-260B c.-471G>A (rs2282688): A-471B; c.-778G>T (rs10034579): A-778B and vice versa. Mutation of a putative PPARγ binding site at −623/−607 upstream of the translation start site was introduced into the haplotype A (pGL4.10 [luc2]–FABP2(A)) and haplotype B (pGL4.10[luc2]–FABP2(B)) containing promoter by exchange of a 4 base core region from CTAT to AGCG named Amut and Bmut, respectively. Introduction of mutations was performed using Quick Change in vitro mutagenesis Kit (Stratagene, La Jolla, CA, USA). All primers were purchased from MWG Biotech AG (Ebersberg, Germany). Sequences of primers are available on request. Verification of constructs was performed by sequencing.

### 2.4. Expression plasmids

The cDNAs of PPARγ2 and RXRα were kindly provided by Dr. T. Weitzel (University Medical Centre Hamburg-Eppendorf, Germany). Cloning procedures were performed using Gateway Technology (Invitrogen) with the pcDNA-Dest40 destination vector as described previously [11,15,16]. The expression plasmids pcDNA3HA-Oct-1 and its empty control vector were a gift from H. Singh (University Chicago, IL, USA).

### 2.5. Transient transfections and reporter assays

Transient transfections were performed with FuGene6 (Roche, Basel, Switzerland) according to the manufacturers instructions. $4\times10^3$ HeLa cells were plated in 96-well plates. Cells were transfected with 30 ng (Figs. 1–2) or 25 ng (Fig. 3) pGL4.10[luc2]–FABP2-Promoter constructs or pGL4.10[luc2] as negative control and 3 ng pGL4.74 [hRluc/TK] vector encoding *Renilla* luciferase as internal control. For co-transfection with PPARγ/RXRα 33 ng (Figs. 1–2) or 25 ng (Fig. 3) Dest40-PPARγ and RXRα expression vectors and 25 ng Dest40 empty control vector (Fig. 3) were used. For co-transfection with Oct-1 25 ng pcDNA3HA-Oct-1 and 75 ng empty pcDNA3HA vector were added. Co-transfection with PPARγ/RXRα/Oct-1 was carried out with 25 ng of each plasmid vector. The PPARγ/RXRα complex was activated 24 h




B

| | Rosi | Fold-change to A - | Fold-change to A + |
|---|---|---|---|
| A | - | 0 | -2.7 |
| | + | 2.7 | 0 |
| A-80B | - | 1.2 | - |
| | + | - | 1.1 |
| A-136B | - | 1.1 | - |
| | + | - | -1.1 |
| A-168B | - | ´1.1 | - |
| | + | - | -1.1 |
| A-260B | - | 1.1 | - |
| | + | - | **-1.5*** ** |
| A-471B | - | -1.3 | - |
| | + | - | **1.4** ** |
| A-778B | - | 1.1 | - |
| | + | - | 1.0 |
| B | - | 1.1 | - |
| | + | - | **2.0*** ** |
| B-471A | - | -1.4 | - |
| | + | - | 1.1 |

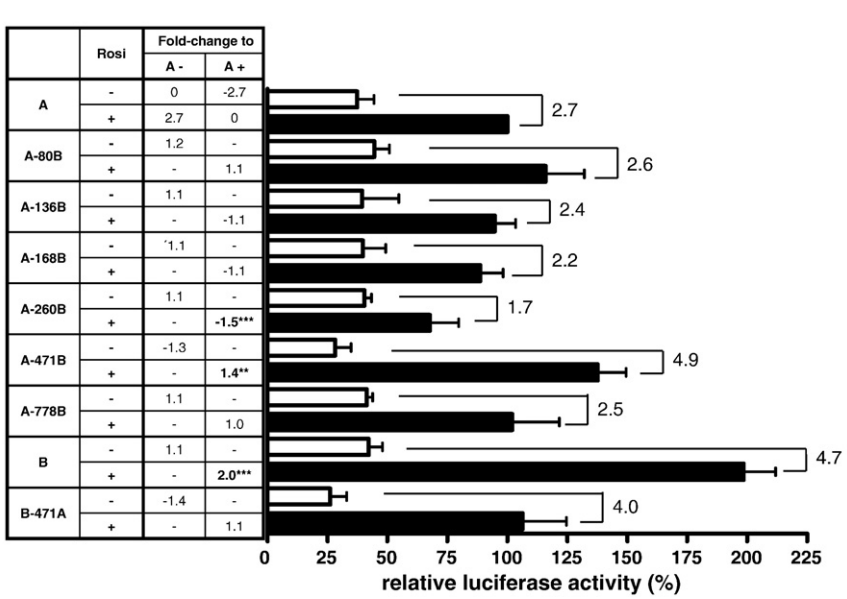



**Fig. 1.** Reporter activities of FABP2 promoter haplotypes and from it derived chimeras in HeLa cells were assessed 48 h after co-transfection with PPARg and/or RXRα and 24 h after activation with rosiglitazone (Rosi) (+) or DMSO control (−) using dual luciferase assay. The *Firefly* luciferase activities were normalized to *Renilla* luciferase activities and are given as relative luciferase activities in % of FABP2 promoter haplotype A. Each experiment was performed in triplicate for each sample. The results are expressed as mean±SEM for three independent experiments. ***$p<0.001$, **$p<0.01$, *$p<0.05$, as compared with haplotype A activity in all figures. (A) Luciferase activities of FABP2 promoter haplotypes (A, B) co-transfected with PPARγ, RXRα or PPARγ/RXRα are shown. (B) Alleles of each polymorphism in the FABP2 promoter haplotypes A>B are given. The positions of polymorphisms c.-80_-79insT (rs5861422), c.-136_-132delAGAGT (rs5861423), c.-168_-166delAAGinsT (rs1973598), c.-260G>A (rs6857641), c.-471G>A (rs2282688), and c.-778G>T (rs10034579) are named as -80, -136, -168, -260, -471, and -778, respectively.