第35回 日本薬物動態学会 奨励賞 受賞講演

遺伝子発現調節機構に着目した薬物動態の個人差要因の解明

Elucidation of the mechanisms of individual differences in pharmacokinetics by focusing on the regulation of gene expression

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35th JSSX Annual Meeting
COI disclosure information

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I have no financial relationship to disclose for our presentation contents.

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Elucidation of the mechanisms of individual differences in pharmacokinetics by focusing on the regulation of gene expression

The regulatory mechanism of expression of pharmacokinetics (PK)-related genes

- genetic polymorphisms
- DNA methylation
- microRNA (miRNA)

DNA methylation

Relationship between DNA Methylation and Interindividual Variability in MATE1 Expression in the Human Liver

Mol Pharmacol. 2018, 93(1):1-7. Tanaka T, Hirota T, Ieiri I.

microRNA

Exosomal miR-328 in plasma, a possible biomarker for estimating BCRP function in the human intestines

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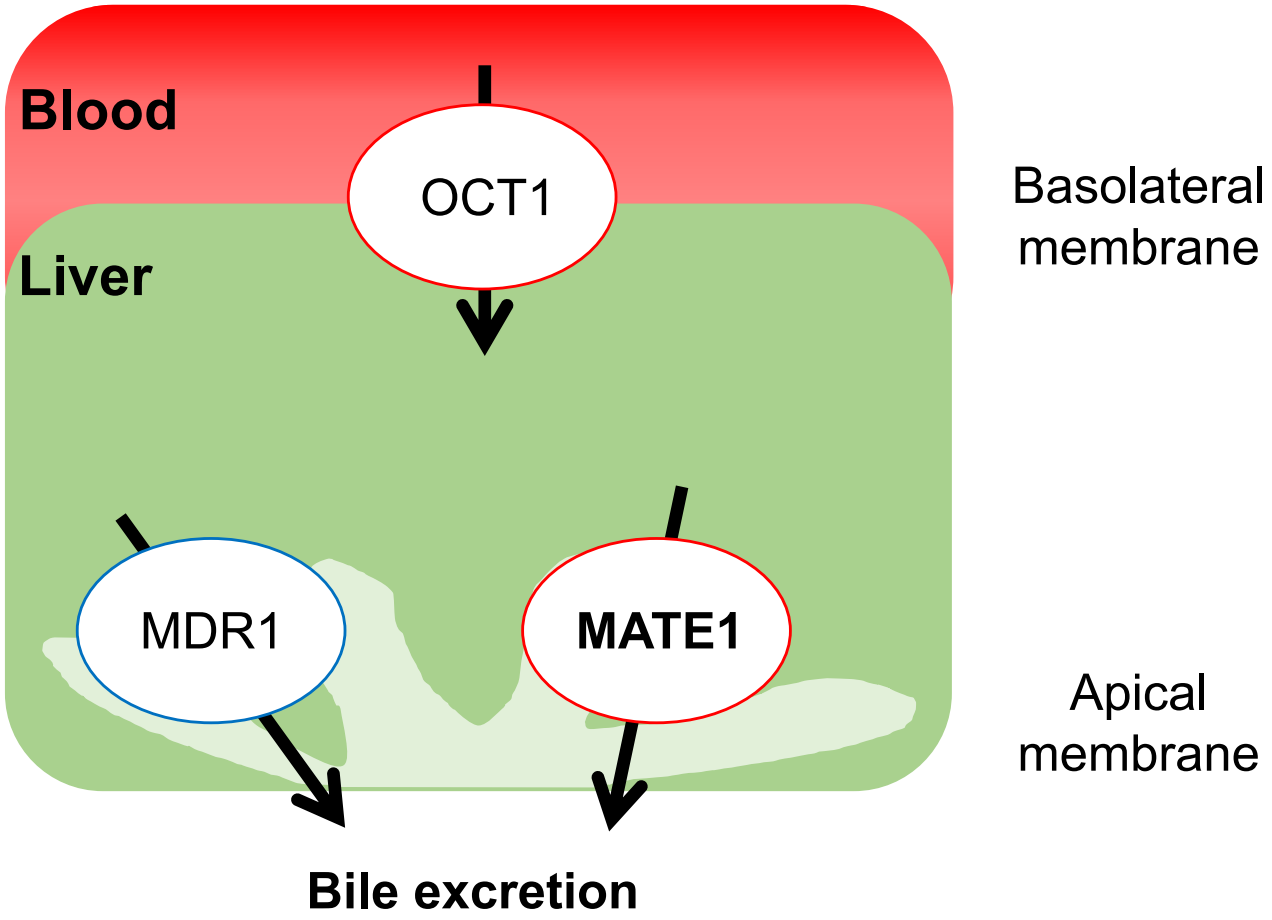
MATE1, which is encoded by SLC47A1, is located in the bile canalicular membrane of the hepatocyte.

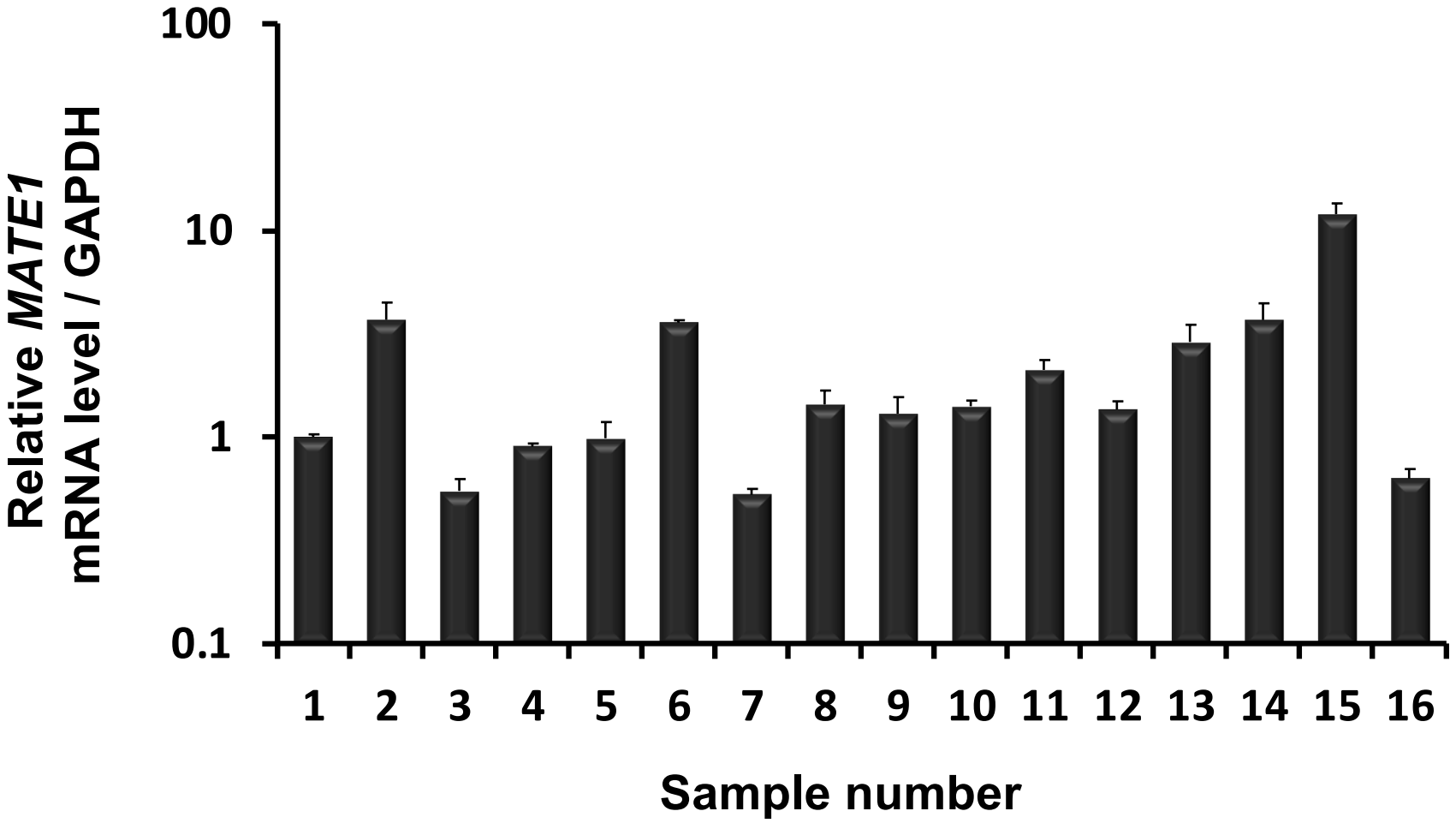
Metformin and cimetidine are typical substrate drugs for MATE1.

Organic cation transport system in human liver



? the contribution of the epigenetic marks on inter-individual variability in hepatic MATE1 levels in human

MATE1 expression in human livers



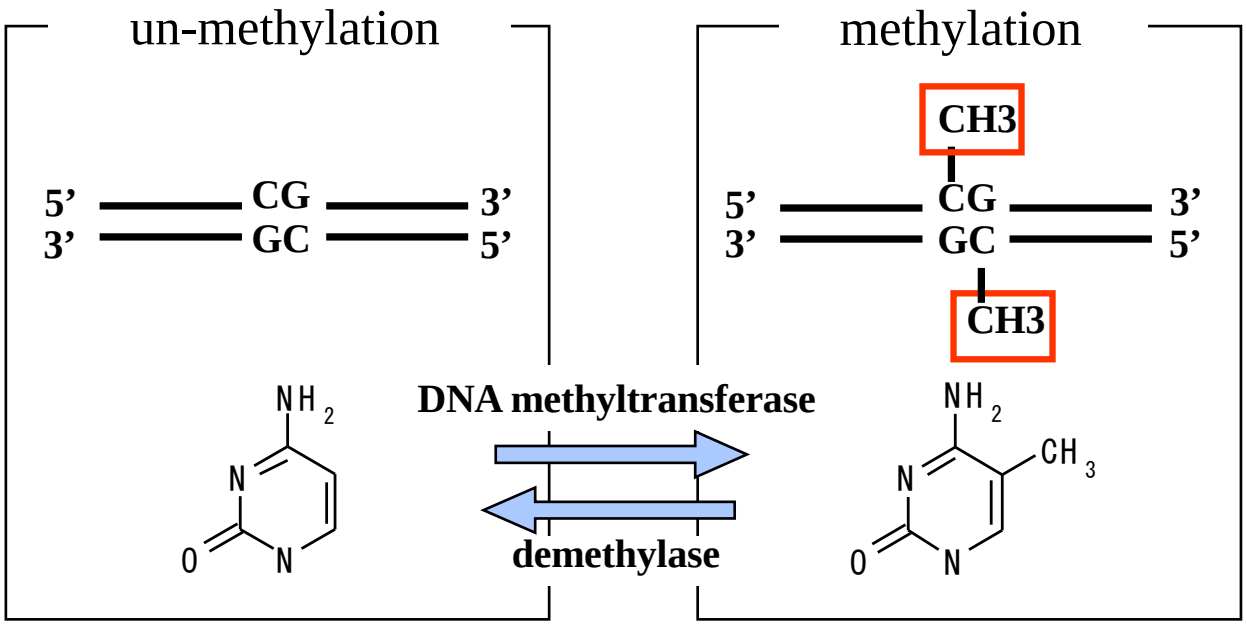
Relative mRNA levels of *MATE1* gene in Caucasian livers (n = 16).
Data represents mean ± S.D. and the results were normalized to the expression of GAPDH.

Inter-individual variability in MATE1 mRNA expression levels were observed.

DNA methylation

- DNA methylation of the 5′-position of cytosine residues is a reversible covalent modification in the palindromic sequence 5′-CpG-3′

- DNA methylation can repress gene transcription either by inhibiting the binding of positive factors to the promoter and/or by recruiting transcriptional co-repressors

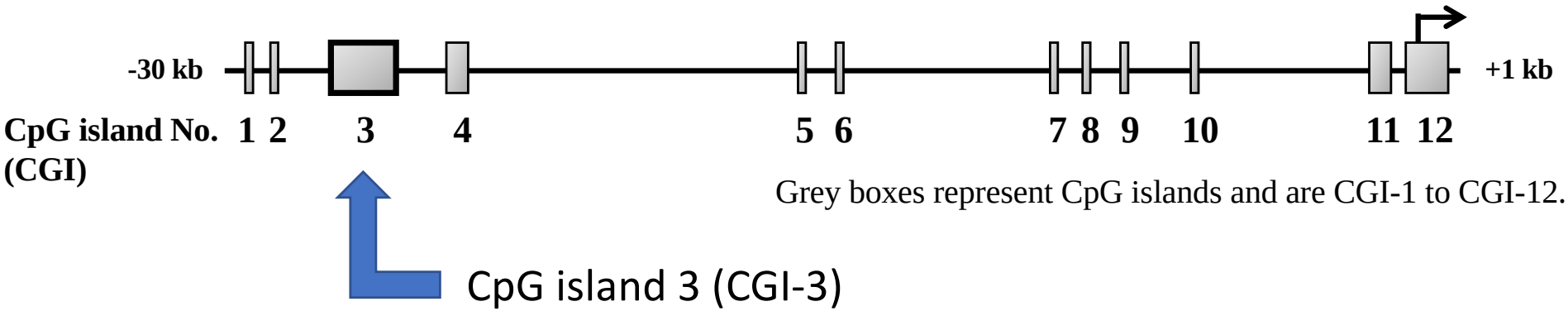


CpG island

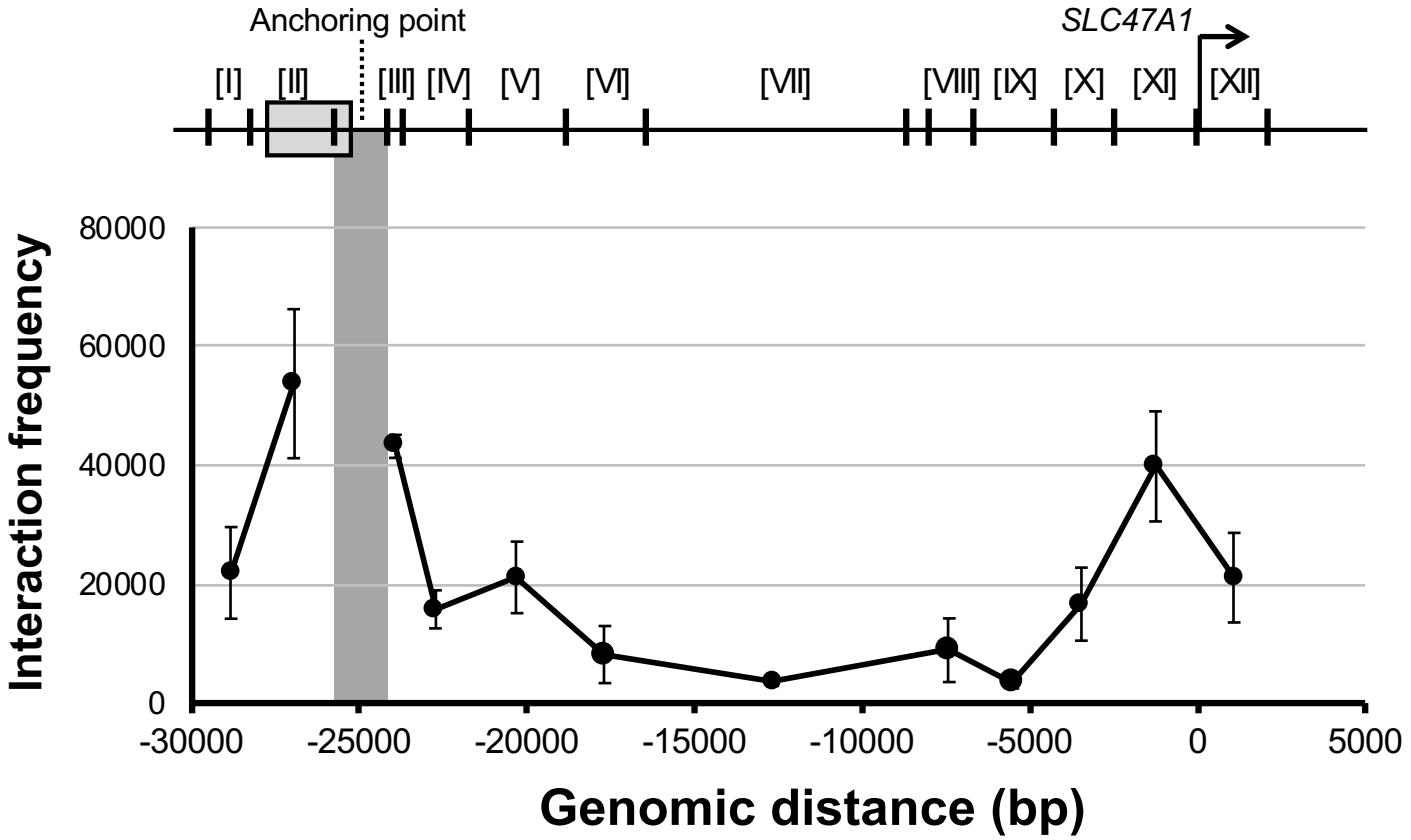
; The regions with a high frequency of cytosine-guanosine dinucleotide sites

- CpG islands are 500 to 2,000 base pair regions rich in CG repeats, present at 5' region in 50% of human genes
- Methylation of cytosine residues in promoter regions and proximal exons of these islands represses the transcription

Schematic of the upstream region of the *SLC47A1* gene



Analysis of long-range DNA interactions between SLC47A1 gene locus and CGI-3



3C assay of the *SLC47A1* gene locus in HepG2 cells.

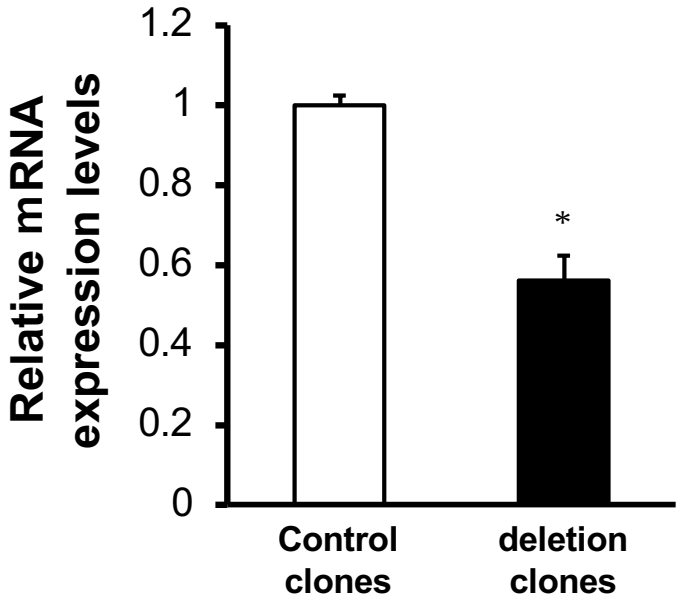
The vertical lines show positions of PstI restriction sites and grey box show CGI-3. Data represents mean ± S.D. (n=3).

The promoter of MATE1 gene interacted strongly with CGI-3.

The long range DNA looping between SLC47A1 gene and CGI-3

The contribution of CGI to MATE1 expression in HepG2 cells

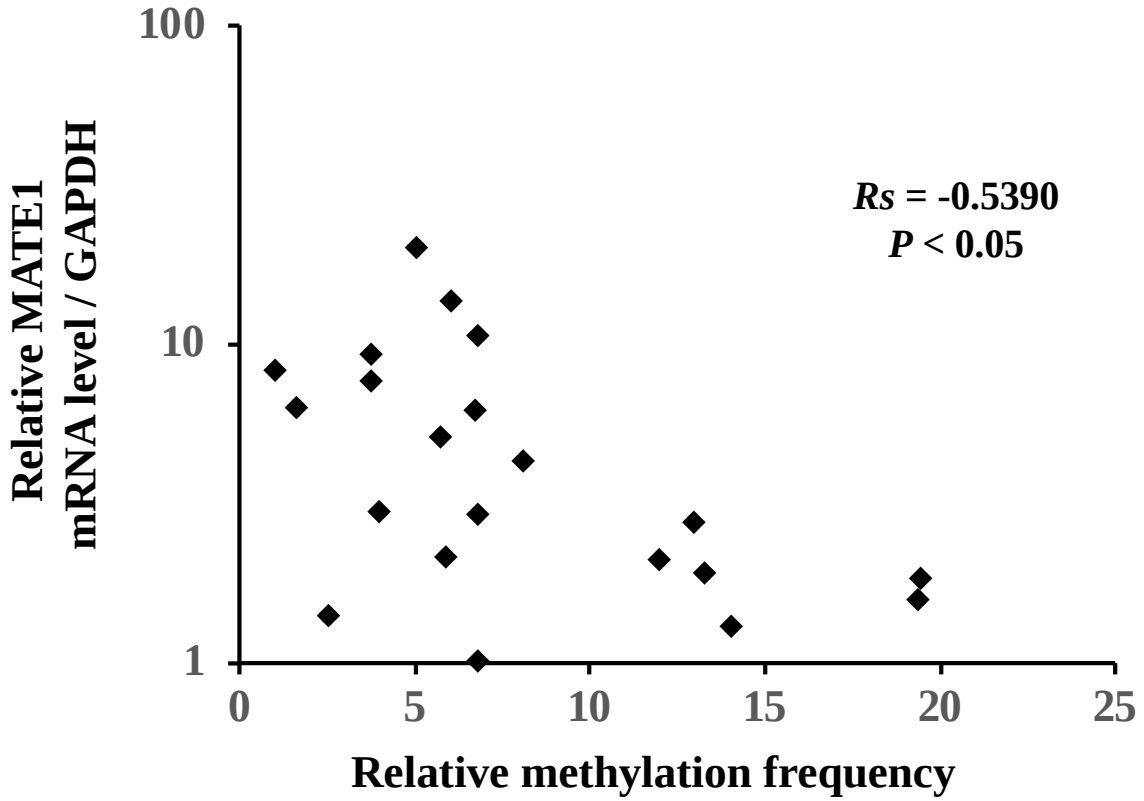
Deletion of the CGI-3 in HepG2 cells using CRISPR/Cas9 system



The effect of deletion of the CpG island on MATE1 mRNA expression in HepG2 cells.
MATE1 mRNA expression was measured by quantitative RT-PCR. Data represents mean ± S.D. of three clones and the results were normalized to the expression of GAPDH. *, $p < 0.05$: statistically analyzed using student t-test.

MATE1 mRNA expression levels in deletion clones were decreased.

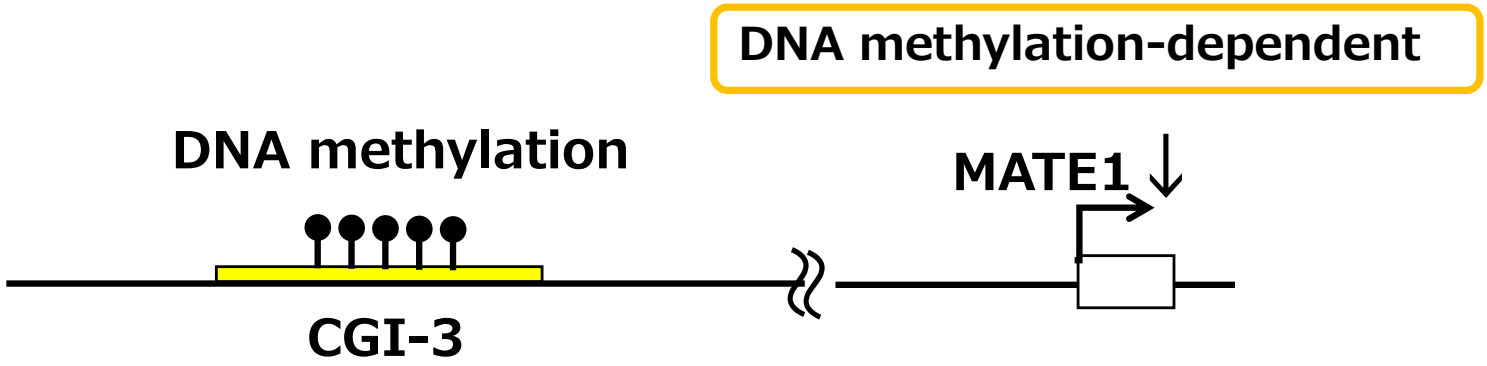
Correlation between the mRNA expression levels of MATE1 and methylation levels of CGI-3



The DNA methylation of CGI-3 was significantly correlated with hepatic MATE1 expression

Conclusion

: DNA Methylation and MATE1 Expression



The DNA methylation in CGI-3 plays an important role in inter-individual differences in hepatic MATE1 expression

DNA methylation

Relationship between DNA Methylation and Interindividual Variability in MATE1 Expression in the Human Liver

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microRNA (miRNA)

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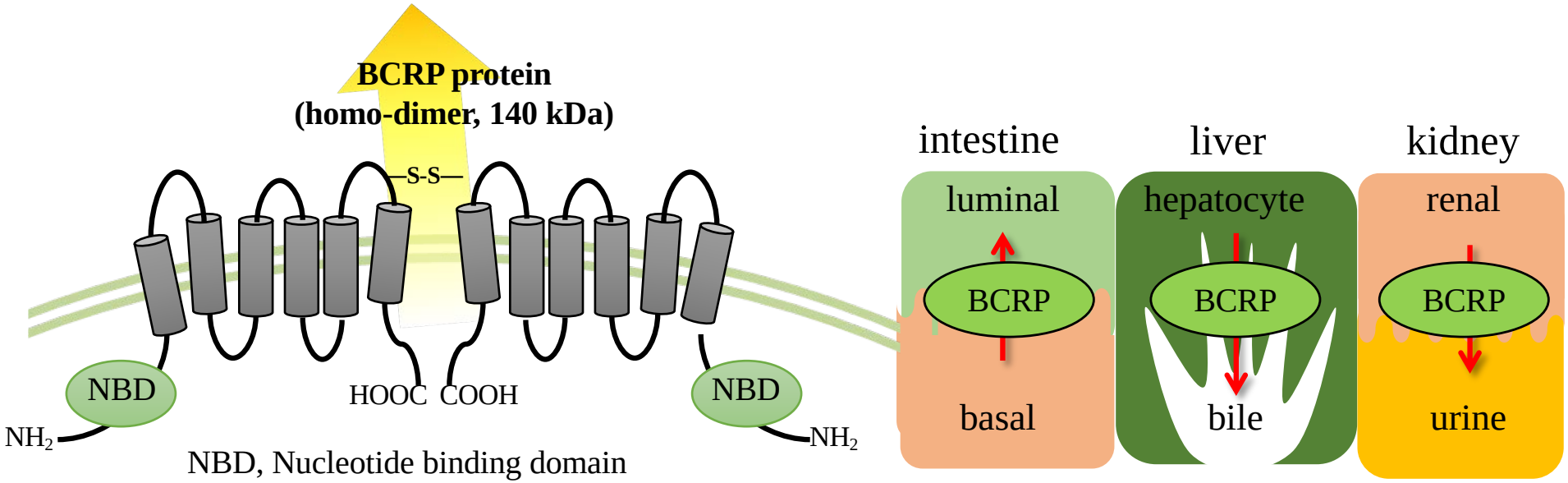
Breast Cancer Resistance Protein (BCRP/ABCG2)

- Breast cancer resistance protein (BCRP) is a member of ATP-binding cassette transporters.
- Substrates of BCRP are mitoxantrone, methotrexate and several TKIs such as imatinib, gefitinib and nilotinib.
- BCRP plays a significant role in absorption, distribution, and elimination of its substrate drugs, and are localized to the apical surface of epithelial cells in intestine, liver, kidney, etc



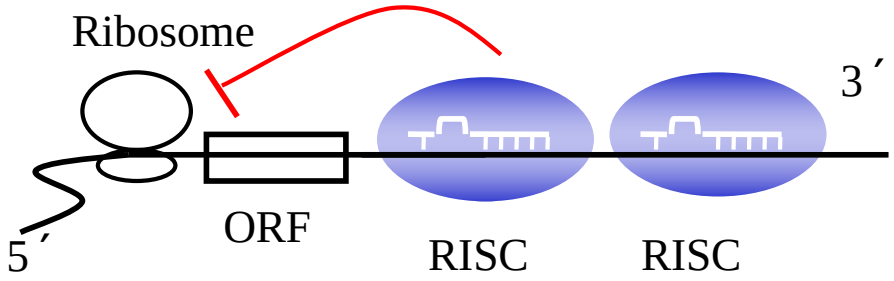
Doyle LA et al, 1998. Robey RW et al, 2009. Zhou S et al., 2001. Jiang XJ et al., 2008

microRNA (miRNA)

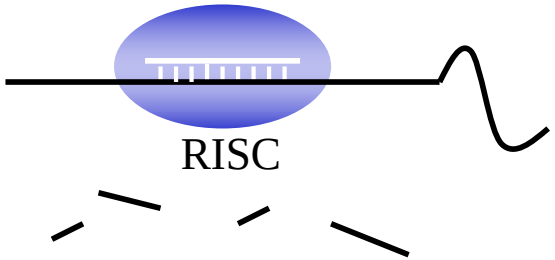
- MicroRNAs (miRNAs) are small noncoding RNAs, approximately 20-25 nucleotides in length, which target the 3'-untranslated region (3'-UTR) of mRNA specifically to prevent translation of mRNA or to degrade mRNA

- MiRNAs involve cellular proliferation, differentiation, and apoptosis in humans, mice and other organisms

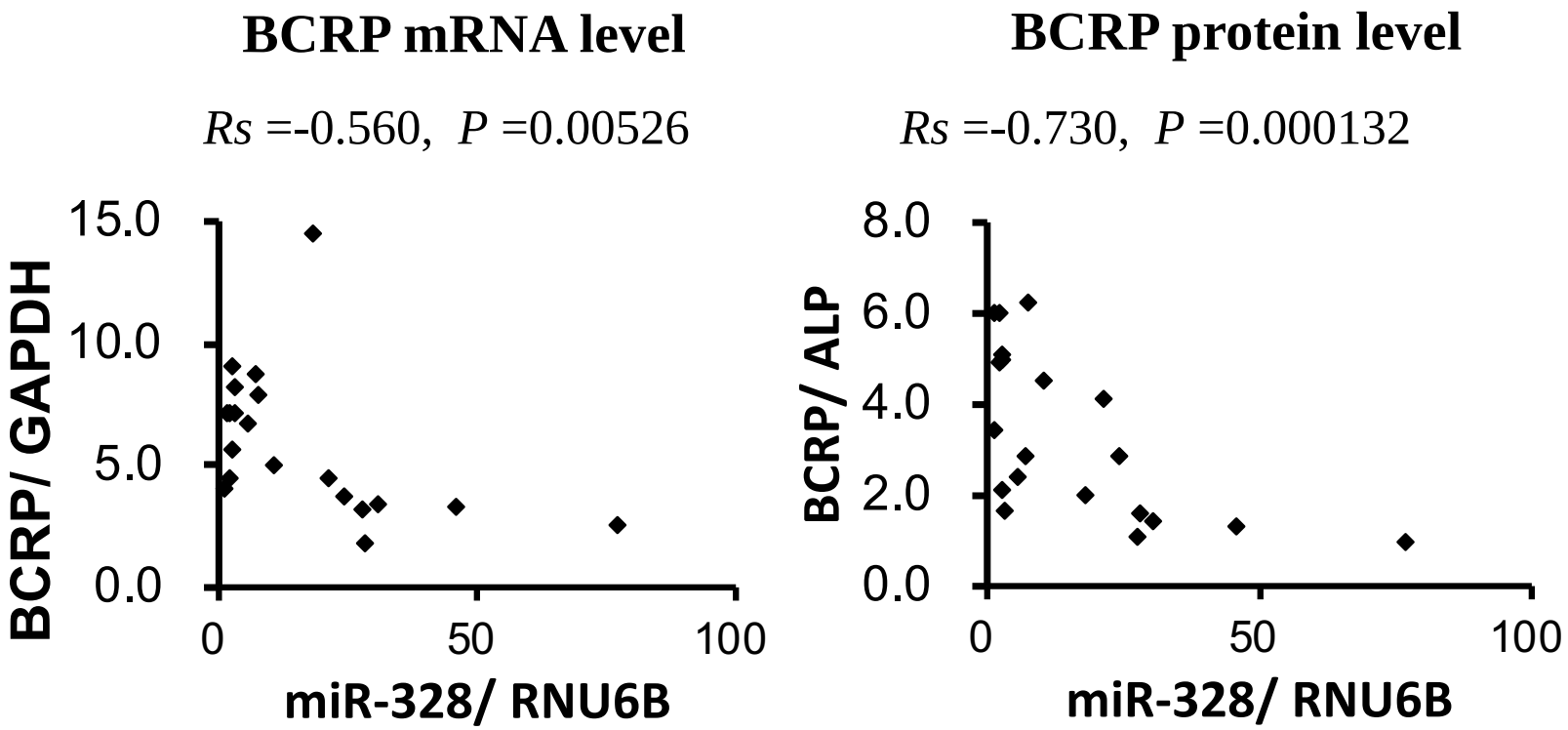
Translational repression



mRNA cleavage



The relationship between miR-328 and BCRP expression in human placentas



Correlation analysis between miR-328 and BCRP genes mRNA or protein expression levels in human placentas samples

421 C/C homozygote samples were selected to eliminate the influence of BCRP genetic variants (n = 20)

Inter-individual difference of miR-328 expression is associated with altered BCRP expression

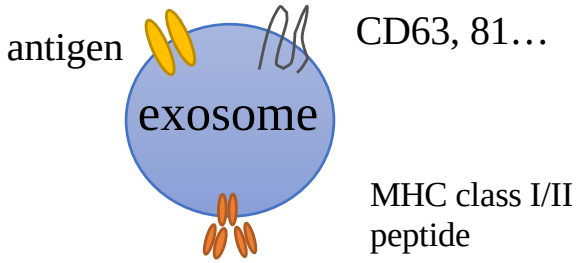
The level of circulating miRNA in plasma as the surrogate biomarker for BCRP function

microRNA –exosomal RNA-

Recent studies demonstrated that miRNAs are secreted from various cells, into body fluids such as blood, urine, breast milk, and saliva via exosomes.

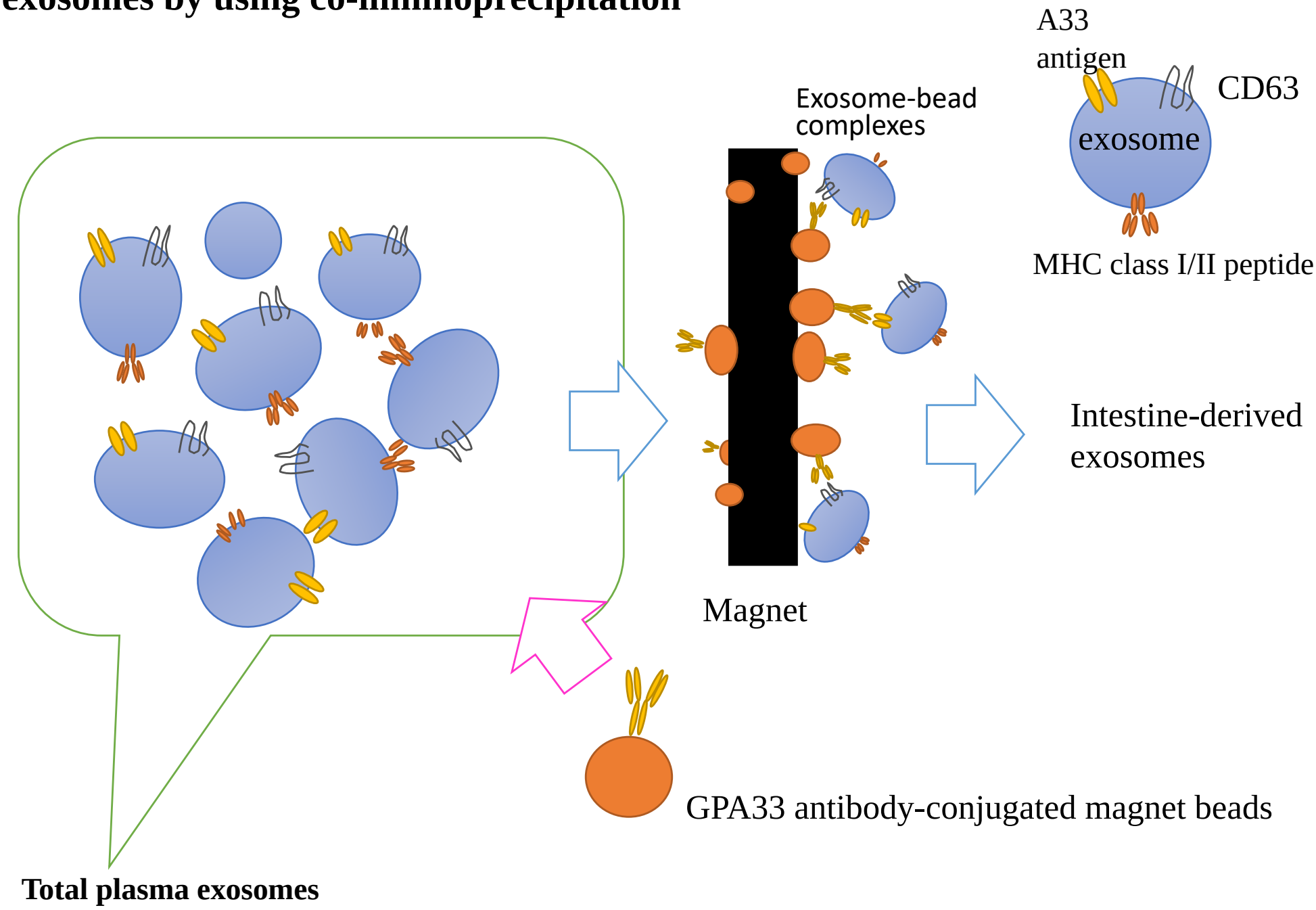
Exosomes are small membrane vesicles of approximately 100 nm that embed protein, lipids, mRNAs, and miRNAs, depending on the origin of the secreting cells.

- Most of the circulating miRNAs are included in lipid or lipoprotein complexes, such as exosomes.
- The circulating exosomal miRNAs were protected from plasma RNase by exosome[1-4)].



1) Gibbings DJ et al.,2009 2) Valadi H et al.,2007 3) Pitati L et al,2010 4) Palaniamy V et al., 2010

Isolation of the intestine-derived exosomes from the total plasma exosomes by using co-immnoprecipitation



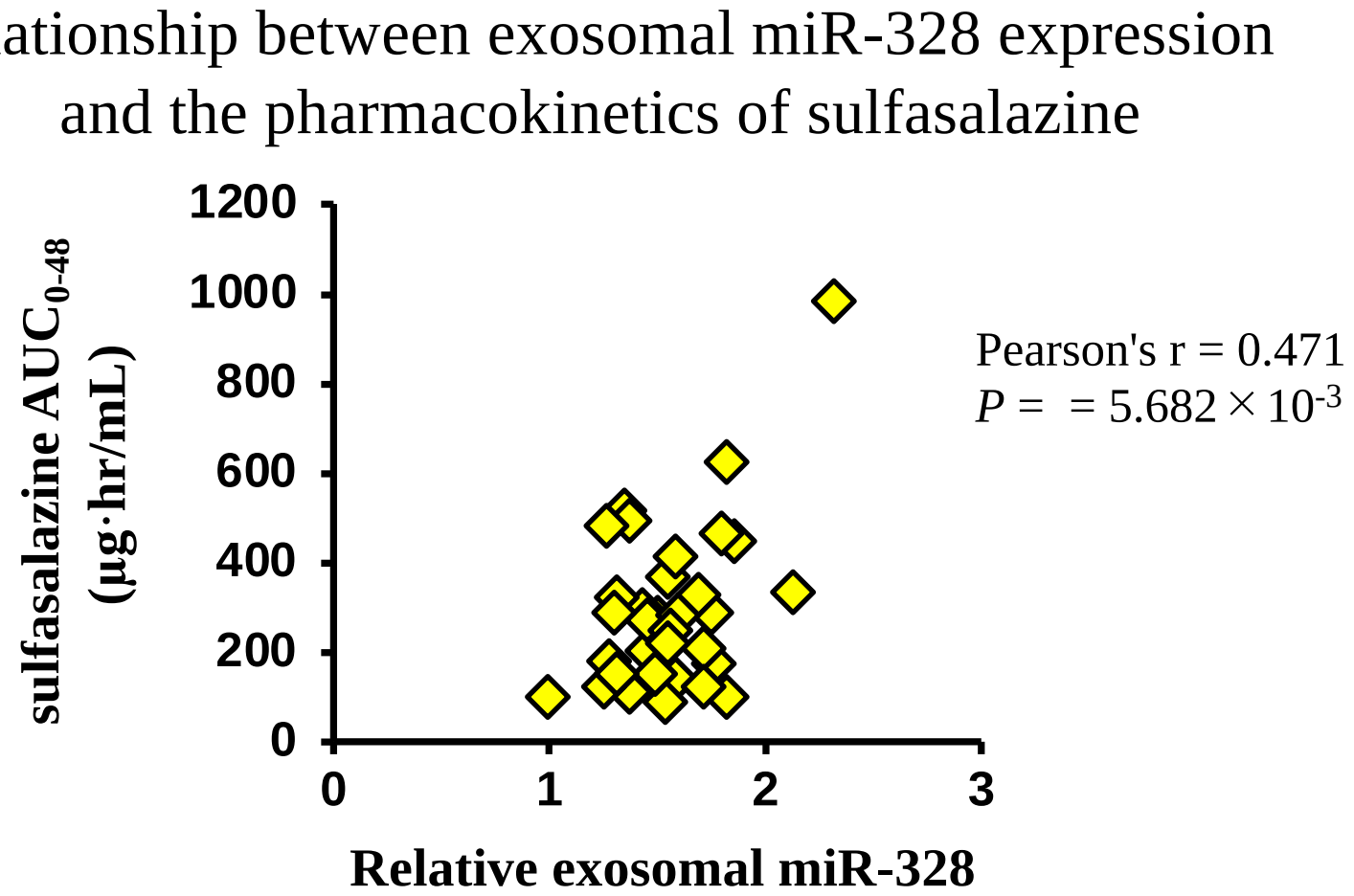
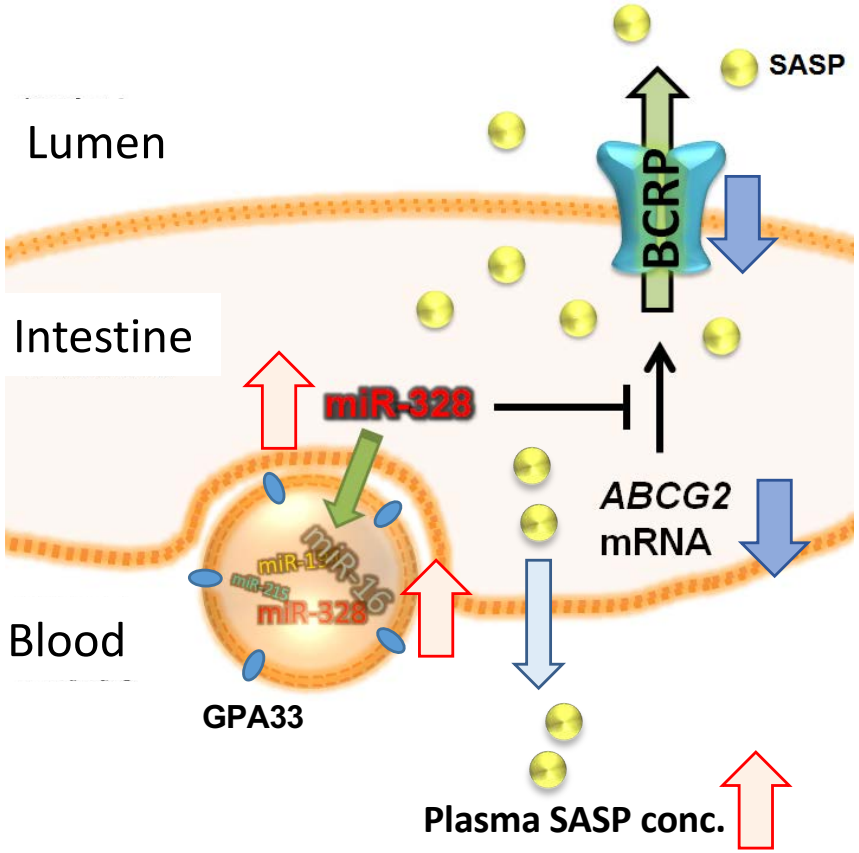


Fig. MiR-328 levels in the intestine-derived exosomes and sulfasalazine AUC in healthy volunteers

Each subject received a single oral dose of 2,000 mg of sulfasalazine. Relative exosomal miR-328 levels were determined the method proposed by Jo Vandesompele.

Conclusion

: Exosomal miR-328 in plasma, a possible biomarker for estimating BCRP function



- MiR-328 suppresses BCRP function
- Decreased BCRP function causes increased the absorption of the drug
- miR-328 expression in intestine can be evaluated by using intestine-derived exosome in blood

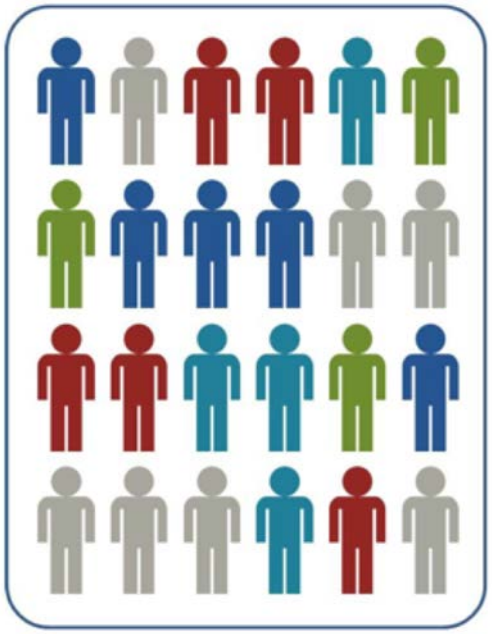
MiR-328 levels in the intestine-derived exosomes can serve as potential biomarkers to predict the pharmacokinetic of the BCRP substrate

Epigenetic changes play an important role in the functions of PK-related genes

A clearer understanding of epigenetic regulation in PK-related genes will provide an insight into novel approaches to individualized drug therapy.

Epigenotype-based prescribing decisions with the aim of maximizing efficacy and mitigating the risks

Patient population



Genotype/Epigenotype -based decisions

Individualized drug therapy