

# Comparative integromics on Angiopoietin family members

YURIKO KATOH<sup>1</sup> and MASARU KATOH<sup>2</sup>

<sup>1</sup>M&M Medical BioInformatics, Hongo 113-0033; <sup>2</sup>Genetics and Cell Biology Section,  
National Cancer Center Research Institute, Tokyo 104-0045, Japan

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**Abstract.** Angiopoietin-1 (ANGPT1), Angiopoietin-4 (ANGPT4), VEGF, FGF2, FGF4, HGF, Ephrin, IL8 and CXCL12 (SFD1) are pro-angiogenic factors (angiogenic activators), while Angiopoietin-2 (ANGPT2), Angiostatin, Endostatin, Tumstatin, Canstatin, THBS1, THBS2, TNFSF15 (VEGI) and Vasohibin (VASH1) are anti-angiogenic factors (angiogenic inhibitors). ANGPT1 and ANGPT2 are ligands for TIE family receptor tyrosine kinases, TIE1 and TIE2 (TEK). Angiopoietin family consists of ANGPT1, ANGPT2, ANGPT4, ANGPTL1 (ANGPT3), ANGPTL2, ANGPTL3 (ANGPT5), ANGPTL4, ANGPTL5, ANGPTL6 and ANGPTL7. TCF/LEF binding sites within the promoter region of human Angiopoietin family members were searched for by using bioinformatics and human intelligence (Humint). Because four TCF/LEF-binding sites were identified within the human *ANGPTL7* promoter, comparative genomics analyses on *ANGPTL7* orthologs were further performed. *ANGPTL7* gene at human chromosome 1p36.22 was located within intron 28 of *FRAP1* gene encoding mTOR protein. Chimpanzee *ANGPTL7* gene, consisting of five exons, was located within NW\_101546.1 genome sequence. Chimpanzee *ANGPTL7* showed 99.4% and 86.1% total-amino-acid identity with human *ANGPTL7* and mouse *Angptl7*, respectively. Human *ANGPTL7* mRNA was expressed in neural tissues, keratoconus cornea, trabecular meshwork, melanotic melanoma and uterus endometrial cancer, while mouse *Angptl7* mRNA was expressed in four-cell embryo, synovial fibroblasts, thymus, uterus and testis. Four TCF/LEF-binding sites within human *ANGPTL7* promoter were conserved in chimpanzee *ANGPTL7* promoter; however, only an unrelated TCF/LEF-binding site occurred in mouse and rat *Angptl7* promoters. Human *ANGPTL7*, characterized as potent target gene of WNT/  $\beta$ -catenin signaling pathway, is a pharmacogenomics target in the fields of oncology and regenerative medicine.

## Introduction

Angiogenesis is regulated by the balance between pro-angiogenic factors (angiogenic activators) and anti-angiogenic factors (angiogenic inhibitors) (1-6). Angiopoietin-1 (ANGPT1), Angiopoietin-4 (ANGPT4), VEGF, FGF2, FGF4, HGF, Ephrin, IL8 and CXCL12 (SFD1) are pro-angiogenic factors, while Angiopoietin-2 (ANGPT2), Angiostatin, Endostatin, Tumstatin, Canstatin, THBS1, THBS2, TNFSF15 (VEGI) and Vasohibin (VASH1) are anti-angiogenic factors (1-17).

ANGPT1, ANGPT2, ANGPT4 are ligands for TIE family receptor tyrosine kinases, TIE1 and TIE2 (TEK) (7-9). ANGPTL1 (ANGPT3), ANGPTL2, ANGPTL3 (ANGPT5), ANGPTL4, ANGPTL5, ANGPTL6 and ANGPTL7 (18-23) are related to ANGPT1, ANGPT2 and ANGPT4. Angiopoietin family consists of ANGPT1, ANGPT2, ANGPT4, ANGPTL1, ANGPTL2, ANGPTL3, ANGPTL4, ANGPTL5, ANGPTL6 and ANGPTL7.

WNT, FGF, Notch and Hedgehog signaling pathways network together during embryogenesis, tissue regeneration and carcinogenesis (24-33). Canonical WNT signals are transduced to the transcriptional complex consisting of TCF/LEF,  $\beta$ -catenin, BCL9/BCL9L and PYGO1/PYGO2 to activate transcription of target genes, such as *DKK1*, *DKK4*, *FGF18* and *FGF20* (34-43); however, WNT-dependent transcriptional regulation of Angiopoietin family members remains unclear.

Here, TCF/LEF binding sites within the promoter region of human Angiopoietin family members were searched for by using bioinformatics and human intelligence (Humint). Because four TCF/LEF-binding sites were identified in the 5'-promoter region of human *ANGPTL7* gene, comparative genomics analyses on *ANGPTL7* orthologs were further performed.

## Materials and methods

**WNT target gene screening.** Genome sequences corresponding to human *ANGPT1*, *ANGPT2*, *ANGPT4*, *ANGPTL1*, *ANGPTL2*, *ANGPTL3*, *ANGPTL4*, *ANGPTL5*, *ANGPTL6* and *ANGPTL7* genes were searched for with BLAST programs (<http://www.ncbi.nlm.nih.gov>) as described previously (44-47). TCF/LEF-binding sites within the 5'-flanking promoter region of the above genes were searched for based on bioinformatics and manual inspection as described previously (38-42,48).

**Identification of chimpanzee and cow *ANGPTL7* orthologs.** Chimpanzee and cow genome sequences homologous to

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Correspondence to: Dr Masaru Katoh, Genetics and Cell Biology Section, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan  
E-mail: mkatoh@ncc.go.jp

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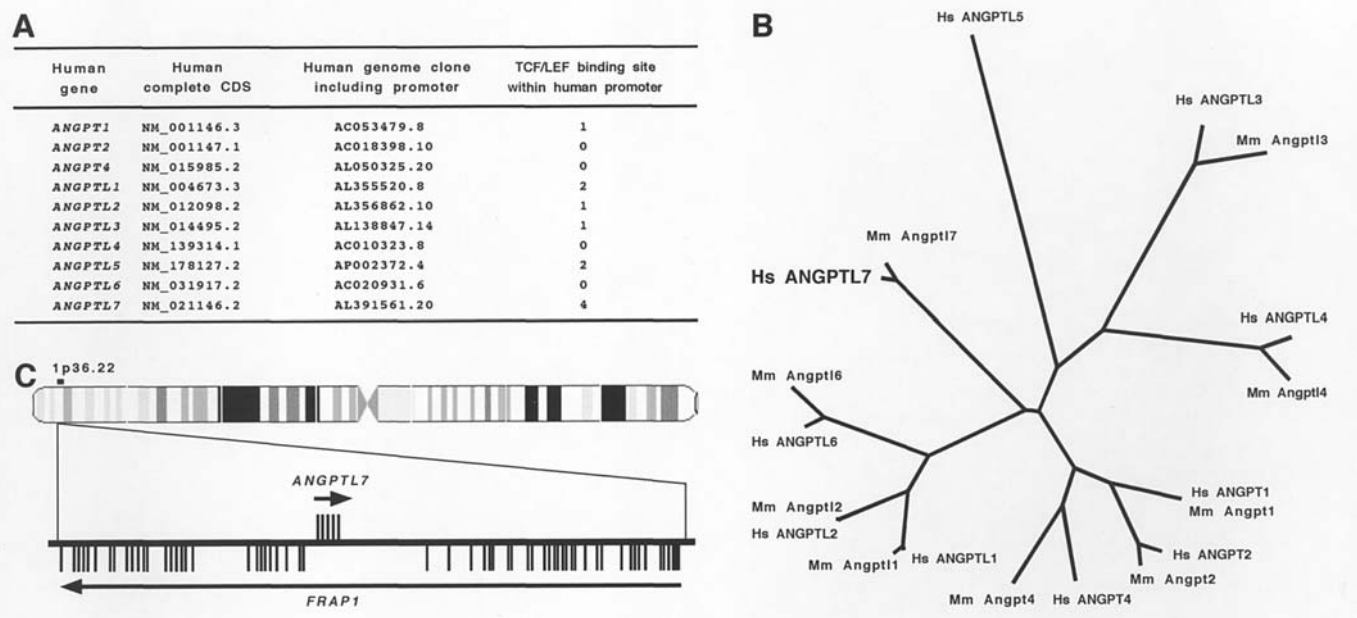


Figure 1. (A), Human Angiopoietin gene family. Gene symbol, complete coding sequence, genome sequence and the number of TCF/LEF-binding sites within promoter region of Angiopoietin family genes are listed. Four TCF/LEF-binding sites exist within the human *ANGPTL7* promoter. (B), Phylogenetic analysis on human and mouse Angiopoietin family members. Hs, human; Mm, mouse. (C), *ANGPTL7* locus at human chromosome 1p36.22. *ANGPTL7* gene, consisting of five exons, is located within intron 28 of the *FRAP1* gene.

human *ANGPTL7* were searched for with BLAST programs as described previously (49-52). TCF/LEF-binding sites within the 5'-flanking promoter region of *ANGPTL7* orthologs were also searched for.

**Comparative proteomics analysis.** Phylogenetic analysis on ANGPT family proteins was performed by using the CLUSTALW program.

**Comparative genomics analyses.** Phylogenetic analysis on promoter of *ANGPTL7* orthologs was performed by using the CLUSTALW program. Promoter region of human and chimpanzee *ANGPTL7* orthologs were aligned by using the Genetyx program and manual curation as described previously (53-56).

**In silico expression analyses.** Expressed sequence tags (ESTs) derived from human *ANGPTL7* gene and mouse *Angptl7* gene were searched for by using the BLAST programs. The sources of human *ANGPTL7* ESTs and those of mouse *Angptl7* ESTs were listed up for *in silico* expression analyses.

Results

**Screening of the TCF/LEF-binding site within promoter region of Angiopoietin family genes.** Human ANGPT1 RefSeq (NM\_001146.3), ANGPT2 RefSeq (NM\_001147.1), ANGPT4 RefSeq (NM\_015985.2), ANGPTL1 RefSeq (NM\_004673.3), ANGPTL2 RefSeq (NM\_012098.2), ANGPTL3 RefSeq (NM\_014495.2), ANGPTL4 RefSeq (NM\_139314.1), ANGPTL5 RefSeq (NM\_178127.2), ANGPTL6 RefSeq (NM\_031917.2) and ANGPTL7 RefSeq (NM\_021146.2) were used as query sequences for the BLAST programs to identify genome clones corresponding to Angiopoietin family genes. The 5'-flanking promoter region of human *ANGPT1*,

*ANGPT2*, *ANGPT4*, *ANGPTL1*, *ANGPTL2*, *ANGPTL3*, *ANGPTL4*, *ANGPTL5*, *ANGPTL6* and *ANGPTL7* genes were identified within AC053479.8, AC018398.10, AL050325.20, AL355520.8, AL356862.10, AL138847.14, AC010323.8, AP002372.4, AC020931.6 and AL391561.20 genome sequences, respectively (Fig. 1A). TCF/LEF-binding sites within the 5'-promoter region of human Angiopoietin family genes were then searched for based on manual inspection. Four TCF/LEF-binding sites were identified within human *ANGPTL7* promoter (Fig. 1A).

**Identification of chimpanzee *ANGPTL7* ortholog.** BLAST programs using human *ANGPTL7* RefSeq revealed that chimpanzee *ANGPTL7* gene was located within NW\_101546.1 genome sequence (Fig. 2A). Exon-intron boundaries of the chimpanzee *ANGPTL7* gene were determined based on the consensus sequence of exon-intron junctions. Chimpanzee *ANGPTL7* gene was found consisting of five exons.

Compared with human *ANGPTL7* RefSeq, one-base insertion occurred at exon 3 of chimpanzee *ANGPTL7* gene within NW\_101546.1 genome sequence. Re-sequencing of the genome sequence around exon 3 of chimpanzee *ANGPTL7* gene should be done in the future to correct the sequencing error.

Complete coding sequence (CDS) of chimpanzee *ANGPTL7* was determined in this study by assembling nucleotide sequences of five exons (Fig. 2A). Genetyx program revealed that nucleotide position 241-1281 was the coding region of chimpanzee *ANGPTL7* complete CDS. Chimpanzee *ANGPTL7* gene was found to encode a 346-amino-acid *ANGPTL7* protein (Fig. 2A).

**Comparative proteomics analysis on *ANGPTL7* orthologs.** Phylogenetic analysis on human and mouse Angiopoietin family members revealed that *ANGPTL7* orthologs were

[illegible]

CTTCAACAGAATCTTTTGATTAGAAATGCCAAGTTGTATAAGGGATACGATAACATCTCAACCTTTAGCAGGCAAAAGAGGCTGGGTATGTGAGAAAACAACATCTGATCCACCTGTATCTCCACTGCTTTTGATTCTGAGAAAAAAA  
 Pt CTTCAACAGAATCTTTTGATTAGAAATGCCAAGTTGTATAAGGGATACGATAACATCTCAACCTTTAGCAGGCAAAAGAGGCTGGGTATGTGAGAAAACAACATCTGATCCACCTGTATCTCCACTGCTTTTGATTCTGAGAAAAAAA  
 \*\*\*\*\*  
 AATTTCTTCAGTAACCTCCAGCTGCCGTAACAGAATATCTTTCTGAGGAGCTAGAAAGCCCTGACTGGAAAAGAGCTCTGGGGGATTATGGCATGGGAGGAGAGCTGTACGGCATCAGGCTTATGTGGGAGGACCCAGCCGCTGACTACAC  
 Hs ---ATTGTCAGTAACCTCCAGCTGCCGTAACAGAATATCTTTCTGAGGAGCTAGAAAGCCCTGACTGGAAAAGAGCTCTGGGGGATTATGGCATGGGAGGAGAGCTGTACGGCATCAGGCTTATGTGGGAGGACCCAGCCGCTGACTACAC  
 Pt ---ATTGTCAGTAACCTCCAGCTGCCGTAACAGAATATCTTTCTGAGGAGCTAGAAAGCCCTGACTGGAAAAGAGCTCTGGGGGATTATGGCATGGGAGGAGAGCTGTACGGCATCAGGCTTATGTGGGAGGACCCAGCCGCTGACTACAC  
 \*\*\*\*\*  
 TCF / LEF  
 =====  
 AGTACAAGTAATCTGCTTGAAGTGGCTCATATACATGGAAGCAATCTGTATGTTCCAAGACAGAGTACTGTTTAAAGACAGAGAACTTTGTATAGATTCTTAGTGAAGAACTTATTTAAGGATGTTGTCTGAAACTCATGTTT  
 Hs AGTACAAGTAATCTGCTTGAAGTGGCTCATATACATGGAAGCAATCTGTATGTTCCAAGACAGAGTACTGTTTAAAGACAGAGAACTTTGTATAGATTCTTAGTGAAGAACTTATTTAAGGATGTTGTCTGAAACTCATGTTT  
 Pt AGTACAAGTAATCTGCTTGAAGTGGCTCATATACATGGAAGCAATCTGTATGTTCCAAGACAGAGTACTGTTTAAAGACAGAGAACTTTGTATAGATTCTTAGTGAAGAACTTATTTAAGGATGTTGTCTGAAACTCATGTTT  
 \*\*\*\*\*  
 TCF / LEF  
 =====  
 TTCACATTCTTTTCAAAGAGCAATGTCCTTCATATATAGTACATGAAAAATACAAATTTTCATGTTTTAGATACAAAGTCATATACAAATAGAGGAGGAATAATTCAAATGCAAACTACACGAAGAAGAGGGAATGAG  
 Hs TTCACATTCTTTTCAAAGAGCAATGTCCTTCATATATAGTACATGAAAAATACAAATTTTCATGTTTTAGATACAAAGTCATATACAAATAGAGGAGGAATAATTCAAATGCAAACTACACGAAGAAGAGGGAATGAG  
 Pt TTCACATTCTTTTCAAAGAGCAATGTCCTTCATATATAGTACATGAAAAATACAAATTTTCATGTTTTAGATACAAAGTCATATACAAATAGAGGAGGAATAATTCAAATGCAAACTACACGAAGAAGAGGGAATGAG  
 \*\*\*\*\*  
 ACTCTAGATATGGATTATTAGGTGACAGGGATTCCTTCTCTATATTTAATATATTTCCAAGTTTGAACACCGGCTAAGGATGGGCCCTCTCTCTGCTCGCTAGAACTGACAGAAAGCATGGGTGACCAATPCAGACATGCT  
 Hs ACTCTAGATATGGATTATTAGGTGACAGGGATTCCTTCTCTATATTTAATATATTTCCAAGTTTGAACACCGGCTAAGGATGGGCCCTCTCTCTGCTCGCTAGAACTGACAGAAAGCATGGGTGACCAATPCAGACATGCT  
 Pt ACTCTAGATATGGATTATTAGGTGACAGGGATTCCTTCTCTATATTTAATATATTTCCAAGTTTGAACACCGGCTAAGGATGGGCCCTCTCTCTGCTCGCTAGAACTGACAGAAAGCATGGGTGACCAATPCAGACATGCT  
 \*\*\*\*\*  
 TCF / LEF  
 =====  
 TGGCACTGCTGAGTTTTCATCTCTGTTGTAATCTTCGACACCTCCCTGCAAAATACAAGTGTTATATGCTAATACTGGCCCTCTCTTTGTTGAAGAGGAAAGCATGTAAAAAGACAACCTGTCAAACCCCTGGGCTACAAAGTCTTTT  
 Hs TGGCACTGCTGAGTTTTCATCTCTGTTGTAATCTTCGACACCTCCCTGCAAAATACAAGTGTTATATGCTAATACTGGCCCTCTCTTTGTTGAAGAGGAAAGCATGTAAAAAGACAACCTGTCAAACCCCTGGGCTACAAAGTCTTTT  
 Pt TGGCACTGCTGAGTTTTCATCTCTGTTGTAATCTTCGACACCTCCCTGCAAAATACAAGTGTTATATGCTAATACTGGCCCTCTCTTTGTTGAAGAGGAAAGCATGTAAAAAGACAACCTGTCAAACCCCTGGGCTACAAAGTCTTTT  
 \*\*\*\*\*  
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 Hs TTGCGCTCTCAAGAGATTACTCTATCTTCTATTCTAGAACTTTCTCAAGTTGGAAGATTCCAATGTGAGATAGTTGTAGATCAAGTGGAGTAAGGATTTATAAAATACAAATATGATCAGAGATGGGAATAATCAGCGTAG  
 Pt TTGCGCTCTCAAGAGATTACTCTATCTTCTATTCTAGAACTTTCTCAAGTTGGAAGATTCCAATGTGAGATAGTTGTAGATCAAGTGGAGTAAGGATTTATAAAATACAAATATGATCAGAGATGGGAATAATCAGCGTAG  
 \*\*\*\*\*  
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 Hs ATAAAAGCATATTACAGCAGATGTCAAATATACAGATCTCATATAACAGCATGGATACATTAACAGGCCCTCCCTGGGTGATAGCGCTCTTTGCGTACCTTAGCGTCACCCCATGGTTATATAACCTTTGAACGGAATTGG  
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 \*\*\*\*\*  
 AAATTCCTGACCCCTCTGCTGCTGCTGATAGATTGAGCCAGTGGCTGGCAGGGGCATAATCTAATAGCGAACTGGAAACACACAATCTCAGCTCTCTTGTCTACCCAGAGACTGTACAGCGAAGGAAGAAAGACTCGGCC  
 Hs AAATTCCTGACCCCTCTGCTGCTGCTGATAGATTGAGCCAGTGGCTGGCAGGGGCATAATCTAATAGCGAACTGGAAACACACAATCTCAGCTCTCTTGTCTACCCAGAGACTGTACAGCGAAGGAAGAAAGACTCGGCC  
 Pt AAATTCCTGACCCCTCTGCTGCTGCTGATAGATTGAGCCAGTGGCTGGCAGGGGCATAATCTAATAGCGAACTGGAAACACACAATCTCAGCTCTCTTGTCTACCCAGAGACTGTACAGCGAAGGAAGAAAGACTCGGCC  
 \*\*\*\*\*  
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 Hs CATCTCCCTCTCTCCCTCTCTCTGCGCTAAGTTGCGCGTGACTTCCACCAACAGGACCGATGACCCCTCCAGATGAGCTGGGAGGGGCTAAGGCCCTGGTGACGCATGGTAGGGGTGGAGGTACAGGCGAGCAACAATATTTAAGATGCTGA  
 Pt CATCTCCCTCTCTCCCTCTCTCTGCGCTAAGTTGCGCGTGACTTCCACCAACAGGACCGATGACCCCTCCAGATGAGCTGGGAGGGGCTAAGGCCCTGGTGACGCATGGTAGGGGTGGAGGTACAGGCGAGCAACAATATTTAAGATGCTGA  
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**Expression profile of human ANGPTL7 and mouse Angptl7 mRNAs.** *In silico* expression analyses were performed to compare the expression profiles of human ANGPTL7 and mouse Angptl7 mRNAs. Human ANGPTL7 mRNA was expressed in neural tissues, keratoconus cornea, trabecular



meshwork, melanotic melanoma and uterus endometrial cancer, while mouse *Angptl7* mRNA was expressed in four-cell embryo, synovial fibroblasts, thymus, uterus and testis.

**Comparative genomics analyses on *ANGPTL7* promoters.** Human and chimpanzee *ANGPTL7* promoters were located within AL391561.20 and NW\_101546.1 genome sequences, respectively, as mentioned above. BLAST programs revealed that cow, mouse and rat *Angptl7* promoters were located within AC174033.2, AC108508.2 and AC125863.3 genome sequences, respectively. Cow *Angptl7* promoter was not used for the following comparative genomics analyses, because one of sequencing gaps within the AC174033.2 genome sequence corresponded to the cow *Angptl7* promoter region.

GC contents of human, chimpanzee, mouse and rat *ANGPTL7* promoters were 41.5%, 41.4%, 50.2% and 50.6%, respectively. GC contents of primate *ANGPTL7* promoters were lower than those of rodent *Angptl7* promoters.

Four TCF/LEF-binding sites within human *VEGFD* promoter were located about 1050, 900, 600, and 550 bp upstream of the transcription start site (Fig. 3). Four TCF/LEF-binding sites within the human *ANGPTL7* promoter were conserved in chimpanzee *ANGPTL7* promoter; however, only an unrelated TCF/LEF-binding site occurred in the mouse and rat *Angptl7* promoters.

## Discussion

TCF/LEF binding sites within the promoter region of human *ANGPT1*, *ANGPT2*, *ANGPT4*, *ANGPTL1*, *ANGPTL2*, *ANGPTL3*, *ANGPTL4*, *ANGPTL5*, *ANGPTL6* and *ANGPTL7* genes were searched for in this study. Because four TCF/LEF-binding sites were identified within the human *ANGPTL7* promoter (Fig. 1A), comparative genomics analyses on *ANGPTL7* orthologs were further performed.

*ANGPTL7* gene at human chromosome 1p36.22 was located within intron 28 of the *FRAP1* gene encoding mTOR protein (Fig. 1C). Chimpanzee *ANGPTL7* gene, consisting of five exons, was located within the NW\_101546.1 genome sequence (Fig. 2A). Chimpanzee *ANGPTL7* showed 99.4% and 86.1% total-amino-acid identity with human *ANGPTL7* and mouse *Angptl7*, respectively (Fig. 2B).

Four TCF/LEF-binding sites within the human *ANGPTL7* promoter were conserved in chimpanzee *ANGPTL7* promoter (Fig. 3); however, only an unrelated TCF/LEF-binding site occurred in the mouse and rat *Angptl7* promoters. GC contents of primate *ANGPTL7* promoters were lower than those of rodent *Angptl7* promoters. Human *ANGPTL7* mRNA was expressed in neural tissues, keratoconus cornea, trabecular meshwork, melanotic melanoma and uterus endometrial cancer, while mouse *Angptl7* mRNA was expressed in four-cell embryo, synovial fibroblasts, thymus, uterus and testis. Together these facts indicate that expression profiles of primate *ANGPTL7* orthologs and rodent *Angptl7* orthologs differ due to promoter evolution.

Human *ANGPTL7*, characterized as a potent target gene of WNT/ $\beta$ -catenin signaling pathway, is a pharmacogenomics target in the fields of oncology and regenerative medicine.

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