

# Two Paralogous Gb3/CD77 Synthases in Birds Show Different Preferences for Their Glycoprotein and Glycosphingolipid Substrates

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**Table S1.** Antibodies used in the experiments.

Antibody	Company
Mouse anti-P1 (clone 650, recognizes Gb3 and P1 antigen)	Ce-Immundiagnostika (Ger-many)
Human anti-P1 (clone P3NIL100, recognizes P1 antigen)	Immucor Inc. (USA)
6xHis-Tag Monoclonal Antibody (HIS.H8)	Thermo Fisher Scientific (USA)
Biotinylated goat anti-mouse IgG/A/M (H/L)	Thermo Fischer Scientific(USA)
Goat anti-Mouse IgG (H+L) Secondary Antibody, AP	Thermo Fisher Scientific (USA)
Biotinylated goat anti-human polyvalent immunoglobulins	Sigma-Aldrich (MERCK) (USA)
Alkaline phosphatase-ExtrAvidin	Sigma-Aldrich (MERCK) (USA)

**Table S2. A.** Primers used for amplification and sequencing of avian *A4GALT*; abbreviations: F: forward primer, R: reverse primer.

Name	Sequence
P_ <i>A4GALT</i> _XhoI_F	5' CTCGAGATGTCCAGCTACCTGCAA 3'
P_ <i>A4GALT</i> _NotI_R	5' GGCGGCCGCTCACATTACAGGCCTTGAC3'

**Table S2. B.** PCR conditions.

Step	Temperature [°C]	Time [hh:mm:ss]	
1. Initial denaturation	98	00:00:30	
2. Denaturation	98	00:00:20	
3. Annealing	55-60	00:00:30	30X
4. Extension/elongation	72	00:00:20	
5. Final elongation	72	00:10:00	

**Table S3.** qPCR conditions, primers and probes used for quantitative analysis of avian *A4GALT* transcripts. Abbreviations: M: enzyme M, P: enzyme P.

Step	Number of Cycles	Temperature [°C]	Time [mm:ss]
Initial denaturation	1	95	10:00
Denaturation	40	95	00:15
Annealing/Extension		60	01:00
qPCR system	7500 Fast		
Reaction type	96-well plate		
Reaction volume [μL]	20		
Forward primer	5' TGTACTGAAGAACTTGAAGAA C	3'	
Reverse primer (P)	5' ATGAACTCATGCTTCGG 3'		
Reverse primer (M)	5' ATGAACTCATGCTTGGG 3'		
Probe (enzyme P)	5' TGCCCTCGGGCTCCAGTCTC 3'		
Probe (enzyme M)	5' TGCCCTTGGTATTGAGTCTC 3'		

P	ATGTCCAGCTACCTGCAAAAAGTACCACAGTGTGCGGAGCCACAGGCCTGGGGCTCTG	60
M	ATGTCCAGCTACCTGCAAAAAGTACCACAGTGTGCGGAGCCACAGGCCTGGGGCTCTG	60
*****		
P	TTTATCCTCATCATTTTCATTTCTGCTCGTTGCCTCTGTTGTGTTCTACCAGAGAACTGGG	120
M	TTTATCCTCATCATTTTCATTTCTGCTCGTTGCCTCTGTTGTGTTCTACCAGAGAACTGGG	120
*****		
P	AAGGACACTGAAGGCCAGCTCTACCACTCGCCTACACAGAACAGGTCTGAAGACTTTTTTG	180
M	AAGGACGCTGAGGGCCAGCTCTATGGCTTGTCTATACAAACAGGTGTAAACAGTTTTTC	180
***** * * * * *		
P	ACTTCTCCTCCCCACACCATTGCTGGTGGGTCCTTCTTCCCCAGGGGATGTGTTTTTT	240
M	GCTTCCCTCCCCACACCATTGCTGGTGGGTCCTTCTTCCCCAGGGGATGTGTTTTTT	240
**** * * * * *		
P	GTGGAGACCTCTGAGCGAATTAAACCAAGTTACCTGTTACGTCGCTCTGTGGAGTCAGCG	300
M	GTGGAGACCTCTGAGCAAACTAACCAAGTTACCTGTTACGTCGCTCTGTGGAGTCAGCG	300
***** * * * * *		
P	GCCCGGGCACACCCTGGAACACGGGTTGTGGTGCTCATGAAAGGCCTGGCAAAGGGGAAT	360
M	GCCCGGGCACACCCTGGAACACGGGTTGTGGTGCTCATGAAAGGCCTGGCAAAGGGGAAT	360
*****		
P	GTCTCATTGCCAGCCACTGGGCATTCTCATTGCTGAGCCGCTTCCCCAACGTGGAGATC	420
M	GTCTCATTGCCAGCCACTGGGCATTCTCATTGCTGAGCTGCTTCCCCAACATGGAGATC	420
***** * * * * *		
P	CAGCCCTGGACTTGGCAGAGCTTTTCTCAGGAACACCTCTGGCAAAGTGGTACTCACAG	480
M	CGGCCCTGGACTTGGCAGAGCTTTTCTCAGGAACACCTCTGGCAAAGTGGTACTCACAG	480
* * * * *		
P	CCTGAGCACCAGAAGGAACCTTATTCTTTCCCGTCTGTCTGACGCCTGCAGAATTACC	540
M	CCTGAGCACCAGAAGGAACCTTATTCTTTCCCGTCTGTCTGACGCCTGCAGAATTGCC	540
***** *		
P	ATCATGTGGAAATTTGGTGGCATCTACCTGGACACAGACTTCATTGTACTGAAGAACTTG	600
M	ATCATGTGGAAATTTGGTGGCATCTACCTGGACACAGACTTCATTGTACTGAAGAACTTG	600
*****		
P	AAGAACCTCACCAATGCCCTCGGGCTCCAGTCTCAGGATGTACTGAATGGGGCCTTCTTG	660
M	AAGAACCTCACCAATGCCCTTGGTATTGAGTCTCAGGATGTGCTGAATGGGGCCTTCTTG	660
***** * * * * *		
P	TCTTTCAAACCGAAGCATGAGTTCATGGAACCTTGCATTACAGACTTTGTGGACAACTAC	720
M	TCCTTCAAACCGAAGCATGAGTTCATAGAACTTGCATGCAGGACTTTGTGATAACTAC	720
** * * * * *		
P	AATGGGTGGATCTGGGCACACCAGGGCCAGAACTGTTAACACGTGTCTTCAAAAAGTTA	780
M	AAAAGCTGGATCTGGGGGCACCAGGGCCACAGCTACTAACACGTGTCTTCAAGAAGTGG	780
** * * * * *		
P	TGCTCCATCAGTAATATCCAGAATGGTATGATCTGCAAAGGAGTGAGTGCTCTTCCCTCT	840
M	TGCTCTATCAGTAATATCCAAAGTGGTATGATCTGCAAAGGAGTGAGTGCTCTTCCCTCT	840
***** * * * * *		
P	GATGCTCTTTATCCCATTCATGGCAGGACTGGAAGAAATTATTTGAAGCAATCAGCTCC	900
M	GATGCTCTTTATCCCATTCATGGCAGGACTGGAAGAAATTATTTGAAGCAATCAGCTCC	900
*****		
P	TCAGAGCTTCACAATCTCCTTAAGAACACCTATGCGGTGCACGTATGGAACAACTGAGC	960
M	TCAGAGCTTCACAATCTCCTTAAGAACACCTATGCGGTGCATGTATGGAACAACTGAGC	960
***** * * * * *		
P	CACGATGCAAGGCTAGAGATCACGTCCCAGGCTTTGCTGGCTCAGCTGTATTCTCAGTTC	1020
M	CACGATGCAAGGCTAGAGATCACGTCCCAGGCTTTGCTGGCTCAGCTGTATTCTCAGTTC	1020
*****		
P	TGCCCTGCCACATCTGCACAGATGAAGAAGGACTTTGAAGAGCAGTCAAGGCCTGTAATG	1080
M	TGCCCTGCCACATCTGCACAGATGAAGAAGGACTTTGAAGAGCAGTCAAGGAACTGTAATG	1080
***** * * * * *		
P	TGA	1083
M	TGA	1083
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**Figure S1.** Comparison of the nucleotide sequences of the *A4GALT* paralogs from the wild pigeon (*C. livia*). Sequences were aligned with ClustalW [1]. ‘P’: sequence deposited in GenBank (NM\_001315524.1), ‘M’: sequence found in our laboratory.

## References

1. McWilliam, H.; Li, W.; Uludag, M.; Squizzato, S.; Park, Y.M.; Buso, N.; Cowley, A.P.; Lopez, R. Analysis Tool Web Services from the EMBL-EBI. *Nucleic Acids Res.* **2013**, *41*, 597–600, doi:10.1093/nar/gkt376.