

Prolonged transition time between colostrum and mature milk in a bear, the giant panda, *Ailuropoda melanoleuca*

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Electronic supplementary material

Figure S1. Altriciality of the neonates of giant pandas.

Table S1. Reproductive history and milk sampling times of giant pandas from whom samples were collected.

Figure S2. Changes in protein profiles of giant panda milk with time after parturition – preparative two-dimensional gels.

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Table S3. Proteins in giant panda milk. Identification of the proteins isolated from bands excised from the one-dimensional gels shown in Figure 3 in the main paper.

Table S4. Relative abundance of the 10 most common small molecules in giant panda milks in colostrum and mature milk, compared with mature phase human and cow milks.

Table S5. Milk oligosaccharides reported in this and previous studies on giant pandas and other species of bear, and their post-partum trends in giant panda milk.

Figure S3. Diversity in the concentration time courses of selected giant panda milk oligosaccharides with time after birth.

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Figure S1. Altriciality of the neonates of giant pandas. Photographs of newly born giant panda cubs with their mothers illustrating both their highly altricial stage of development, the relative size of mothers and cubs, and thus the considerable degree of maternal care required. Images courtesy of Chengdu Research Centre for Giant Panda Breeding.

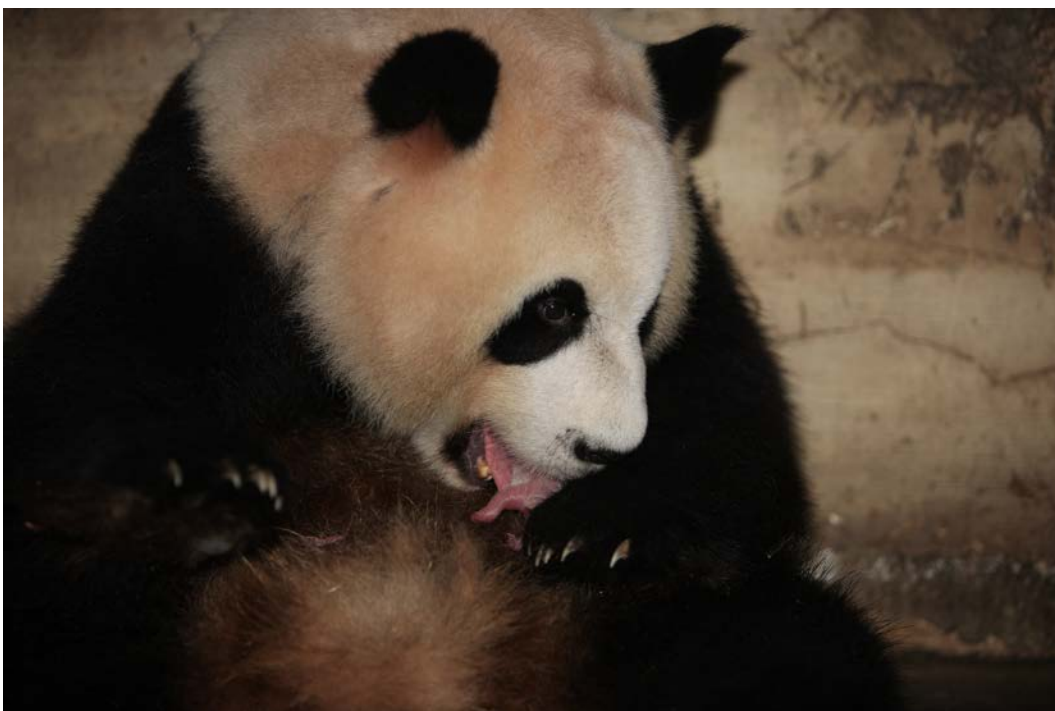


Table S1. Reproductive history and milk sampling times of giant pandas from whom samples were collected. All mothers and cubs were born in captivity. ISN, International Studbook Number. Parturition dates in bold indicate the beginning of the lactation period from which samples were collected.

| Name, ISN & date of birth | Parturition history | Milk sample collection dates | Days post-partum |
|------------------------------|--|------------------------------|------------------|
| Ya Ya 362 24 Aug 1990 | 9 Sept 1997, twins; 1 died same day | 13 Jan 2007 | 147 |
| | | 18 Jan 2007 | 152 |
| | 9 Sept 1999, twins | 23 Jan 2007 | 157 |
| | 20 Aug 2001, twins | 29 Jan 2007 | 163 |
| | 28 Aug 2002, twins | 31 Jan 2007 | 165 |
| | 6 Sept 2003, single | 07 Feb 2007 | 172 |
| | 30 Aug 2005, single | | |
| | 19 Aug 2006, single | | |
| 24 Aug 2008, single | | | |
| 10 Sept 2010, single | | | |
| <hr/> | | | |
| Li Li 387 03 Sept 1992 | 12 July 2001, twins, died 19 Sept & 17 Oct, 2001 | 07 Aug 2007 | 4 |
| | 08 July 2002, single, died 08 July 2002 | 25 July 2011 | 1 |
| | 03 Aug 2007, single, died same day | 26 July 2011 | 2 |
| | 19 July 2009, twins | 28 July 2011 | 4 |
| | 24 July 2011, single | 29 July 2011 | 5 |
| | 28 July 2012, twins, 1 died same day | 30 July 2011 | 6 |
| | | 31 July 2011 | 7 |
| | | 01 Aug 2011 | 8 |
| | | 02 Aug 2011 | 9 |
| | | 03 Aug 2011 | 10 |
| | | 05 Aug 2011 | 12 |
| | | 06 Aug 2011 | 13 |
| | | 07 Aug 2011 | 14 |
| | | 08 Aug 2011 | 15 |
| | | 09 Aug 2011 | 16 |
| | | 10 Aug 2011 | 17 |
| | | 11 Aug 2011 | 18 |
| | | 12 Aug 2011 | 19 |
| | | 14 Aug 2011 | 21 |
| | | 15 Aug 2011 | 22 |
| | | 29 July 2012 | 1 |
| | | 30 July 2012 | 2 |
| | 31 July 2012 | 3 | |
| | 1 Aug 2012 | 4 | |
| | 2 Aug 2012 | 5 | |
| | 3 Aug 2012 | 6 | |
| | 4 Aug 2012 | 7 | |
| | 5 Aug 2012 | 8 | |
| | 6 Aug 2012 | 9 | |
| | 8 Aug 2012 | 11 | |
| | 9 Aug 2012 | 12 | |

| Name, ISN & date of birth | Parturition history | Milk sample collection dates | Days post-partum |
|----------------------------------|--|--|---|
| | | 10 Aug 2012 | 13 |
| | | 11 Aug 2012 | 14 |
| | | 12 Aug 2012 | 15 |
| | | 13 Aug 2012 | 16 |
| | | 15 Aug 2012 | 18 |
| | | 16 Aug 2012 | 19 |
| Shu Qing 480 03 Aug 1999 | 26 Aug 2004, single 05 July 2007, twins 04/05 Aug 2008, twins | 08 July 2007 20 Aug 2007 10 Dec 2007 | 3 46 158 |
| Qi Zhen 490 04 Sept 1999 | 07 Aug 2006, twins 10 Aug 2008, twins 06 Aug 2010, twins 04 Aug 2011, twins | 12 Aug 2006 14 Aug 2006 13 Aug 2006 15 Aug 2006 10 Jan 2007 11 Jan 2007 14 Jan 2007 16 Jan 2007 | 5 7 6 8 156 157 160 162 |
| Yuan Yuan 561 01 Aug 2003 | 06 Sept 2008, single 15 Aug 2010, twins 25 Aug 2012, twins, 1 died same day | 26 Aug 2012 27 Aug 2012 28 Aug 2012 29 Aug 2012 01 Sept 2012 04 Sept 2012 05 Sept 2012 08 Sept 2012 09 Sept 2012 10 Sept 2012 13 Sept 2012 16 Sept 2012 17 Sept 2012 19 Sept 2012 20 Sept 2012 21 Sept 2012 23 Sept 2012 24 Sept 2012 25 Sept 2012 01 Oct 2012 10 Oct 2012 | 1 2 3 4 7 10 11 14 15 16 19 22 23 25 26 27 29 30 31 37 46 |
| Xiao Yatou 635 13 Aug 2006 | 12 Aug 2012, single | 16 Aug 2012 24 Aug 2012 25 Aug 2012 26 Aug 2012 | 4 12 13 14 |

| Name, ISN & date of birth | Parturition history | Milk sample collection dates | Days post-partum |
|---------------------------|---------------------|------------------------------|------------------|
| | | 27 Aug 2012 | 15 |
| | | 30 Aug 2012 | 17 |
| | | 03 Sept 2012 | 22 |
| | | 13 Sept 2012 | 32 |
| | | 14 Sept 2012 | 33 |
| | | 17 Sept 2012 | 36 |
| | | 18 Sept 2012 | 37 |
| | | 19 Sept 2012 | 38 |
| | | 20 Sept 2012 | 39 |
| | | 21 Sept 2012 | 40 |
| | | 30 Sept 2012 | 49 |
| | | 01 Oct 2012 | 50 |
| | | 09 Oct 2012 | 58 |

Figure S2. Changes in protein profiles of giant panda milk with time after parturition – preparative two-dimensional gels. Preparative two-dimensional gel electrophoresis of milk sampled on day1 (A; panda Li Li) and on day 152 (B; panda Ya Ya). The numbered protein spots were excised and subjected to tryptic digestion and mass spectrometry, after which peptide mass fingerprints were used to interrogate the giant panda genome with MASCOT software. Identifications of the numbered protein spots and comments are given in table S2. These identifications were combined with identifications from bands excised from 1-D gels to provide the protein identifications in Figure 3 of the main paper and the associated tables 1 and S3. First dimension (IEF) on IPG strips pH 3–10 and the second dimension on 4–12% gradient SDS-PAGE gels. The protein spots were visualized by Coomassie blue staining.

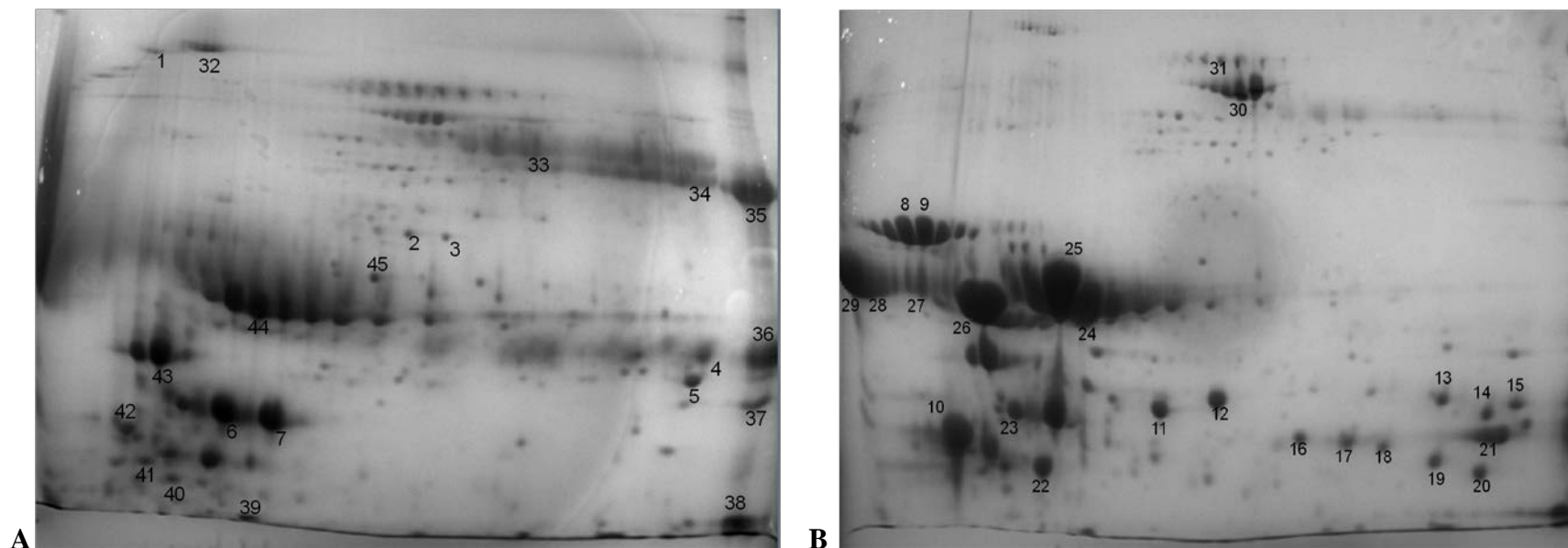


Table S2. Identification of the proteins isolated from bands excised from the two-dimensional gels shown in Figure S2. Milks sampled from panda Li Li 1 day and from Ya Ya 152 days after parturition. The putative functions and comments are drawn from a variety of sources including NCBI and UniProtKB/Swiss-Prot databases.

| Spot number (day of sample) ^a | Protein ^b | Accession number ^c | Number of matches (unique peptides) ^d | Mascot score ^e | Putative function and comments ^f |
|--|-----------------------------------|-------------------------------|--|---------------------------|---|
| 1 (1) | Bile salt-activated lipase | gi 301777998 | 67(36) | 1286 | Presumed to assist with digestion of lipids, triglycerides in particular. |
| 2 (1) | Polymeric immunoglobulin receptor | gi 301767800 | 230(122) | 4557 | Receptor for IgA and IgM mediating secretion, part of which (secretory piece) binds to IgA to protect it against proteolytic cleavage in intestine. |
| 3 (1) | Polymeric immunoglobulin receptor | gi 301767800 | 230(122) | 4160 | Receptor for IgA and IgM mediating secretion, part of which (secretory piece) binds to IgA to protect it against proteolytic cleavage in intestine. |
| 4 (1) | Immunoglobulin λ^* | gi 281339129 | 176(70) | 3098 | Light chain isoform associated with all immunoglobulin classes. |
| 5 (1) | β -casein | gi 301768260 | 86(36) | 905 | Phosphoprotein. Source of amino acids, delivers calcium, phosphate, lipids. Determines the surface properties of the casein micelles. |
| 6 (1) | β -lactoglobulin-1 | gi 301778529 | 68(30) | 584 | Binds and probably transports retinol (vitamin A), vitamin D, fatty acids including PUFAs ^g . |
| 7 (1) | β -lactoglobulin-1 | gi 301778533 | 150(64) | 2843 | Binds and probably transports retinol (vitamin A), vitamin D, fatty acids including PUFAs ^g . |
| 8 (152) | β -lactoglobulin-1 | gi 301778529 | 71(24) | 462 | Binds and probably transports retinol (vitamin A), vitamin D, fatty acids including PUFAs ^g . |
| 9 (152) | Keratin | gi 301779149 | 26(16) | 639 | Structural constituent of epidermis. |
| 10 (152) | Whey acidic protein | gi 301777338 | 93(41) | 1247 | Function unclear. Innate immunity? Regulates proliferation of mammary epithelial cells? |
| 11 (152) | β -lactoglobulin-2 | gi 301778531 | 276(140) | 4222 | Binds and probably transports retinol (vitamin A), vitamin D, fatty acids including PUFAs ^g . |

| | | | | | |
|----------|--|------------------------------|--------------------|-------------|--|
| 12 (152) | β -lactoglobulin-2 β -lactoglobulin-1 | gi 301778531 gi 301778533 | 275(157) 71(32) | 4850 923 | Bind and probably transport retinol (vitamin A), vitamin D, fatty acids including PUFAs ^g . |
| 13 (152) | β -lactoglobulin-2 | gi 301778531 | 125(2) | 131 | Binds and probably transports retinol (vitamin A), vitamin D, fatty acids including PUFAs ^g . |
| 14 (152) | Keratin | gi 301779149 | 19(11) | 414 | Structural constituent of epidermis. |
| 15 (152) | Keratin | gi 301779149 | 22(14) | 467 | |
| 16 (152) | Anti-leukoproteinase | gi 301764004 | 83(27) | 663 | |
| 17 (152) | Anti-leukoproteinase | gi 301764004 | 64(16) | 320 | Acid-stable proteinase inhibitor with strong affinities for trypsin, chymotrypsin, elastase, and cathepsin G. |
| 18 (152) | Keratin | gi 301779149 | 19(10) | 458 | Structural constituent of epidermis. |
| 19 (152) | Keratin | gi 301779149 | 12(8) | 357 | |
| 20 (152) | Keratin | gi 301779149 | 11(7) | 173 | |
| 21 (152) | Anti-leukoproteinase | gi 301764004 | 31(11) | 289 | Acid-stable proteinase inhibitor with strong affinities for trypsin, chymotrypsin, elastase, and cathepsin G. |
| 22 (152) | α -lactalbumin- 2 | gi 301783681 | 87(40) | 1037 | Regulatory subunit of lactose synthase. Changes the substrate specificity of galactosyltransferase making glucose a good acceptor substrate for this enzyme enabling LS to synthesize lactose. |
| 23 (152) | β -lactoglobulin-1, | gi 301778533 | 60(23) | 575 | Binds and probably transports retinol (vitamin A), vitamin D, fatty acids including PUFAs ^g . |
| | α -lactalbumin-2 | gi 301783681 | 15(6) | 137 | Regulatory subunit of lactose synthase. Changes the substrate specificity of galactosyltransferase making glucose a good acceptor substrate for this enzyme enabling LS to synthesize lactose. |
| 24 (152) | β -casein | gi 301768260 | 73(18) | 516 | Phosphoprotein. Source of amino acids, delivers calcium, phosphate, lipids. Determines the surface properties of the casein micelles. |
| 25 (152) | β -casein | gi 301768260 | 67(19) | 508 | |
| 26 (152) | β -casein | gi 301768260 | 23(7) | 230 | |
| 27 (152) | β -casein | gi 301768260 | 4(2) | 92 | |
| 28 (152) | Keratin | gi 301775745 | 2(2) | 108 | Structural constituent of epidermis. |

| | | | | | |
|----------|-------------------------------------|--------------|----------|------|---|
| 29 (152) | κ -casein | gi 281347661 | 3(2) | 79 | Stabilizes micelle formation, preventing casein precipitation in milk. |
| 30 (152) | Serum albumin | gi 301786252 | 243(127) | 3508 | Main protein of plasma. Binds Ca^{2+} , Na^+ , K^+ , fatty acids, hormones, bilirubin and drugs. Major zinc transporter of plasma. |
| 31 (152) | Polymeric immunoglobulin receptor | gi 301767800 | 59(25) | 876 | Receptor for IgA and IgM mediating secretion, part of which (secretory piece) binds to IgA to protect it against proteolytic cleavage in intestine. |
| 32 (1) | Bile salt-activated lipase | gi 301777998 | 66(34) | 1033 | Also termed stimulated- or dependent-lipase. Presumed to assist with digestion of lipids, triglycerides in particular. |
| 33 (1) | Immunoglobulin α | gi 62183972 | 33(15) | 346 | Heavy chain of IgA. Secretory antibody. Abundant in secretions (e.g. tears, saliva, bile, milks). |
| 34 (1) | Immunoglobulin γ | gi 62183980 | 33(16) | 520 | Heavy chain of immunoglobulin G (IgG). Antibody. Abundant in serum, less so in secretions. |
| | Lactadherin | gi 301768371 | 34(11) | 445 | Maintains intestinal epithelial homeostasis and the promotion of mucosal healing. |
| 35 (1) | Immunoglobulin γ heavy chain | gi 62183978 | 74(38) | 1038 | Immunoglobulin G (IgG). Antibody. Abundant in serum, less so in secretions. |
| | Lactadherin | gi 301768371 | 20(13) | 413 | Maintains intestinal epithelial homeostasis and the promotion of mucosal healing. |
| 36 (1) | Immunoglobulin λ^* | gi 281339129 | 37(18) | 676 | Light chain isoform associated with all immunoglobulin subclasses. |
| 37 (1) | No confident prediction | | | | |
| 38 (1) | Lysozyme C | gi 301783675 | 34(15) | 527 | Milk isoform of lysozyme. Anti-bacterial. |
| 39 (1) | α -lactalbumin-2 | gi 301783681 | 43(23) | 662 | Regulatory subunit of lactose synthase. Changes the substrate specificity of galactosyltransferase, making glucose a good acceptor substrate for this enzyme and enabling LS to synthesize lactose. |

| | | | | | |
|--------|--------------------------|--------------|---------|-----|--|
| 40 (1) | α -lactalbumin-1, | gi 301783679 | 14(11) | 286 | Regulatory subunit of lactose synthase. Changes the substrate specificity of galactosyltransferase making glucose a good acceptor substrate for this enzyme enabling LS to synthesize lactose. |
| | β -casein, | gi 301768260 | 35(7) | 165 | Phosphoprotein. Source of amino acids, delivers calcium, phosphate, lipids. Determines the surface properties of the casein micelles. |
| | β -lactoglobulin-1 | gi 301778529 | 7(4) | 116 | Binds and probably transports retinol (vitamin A), vitamin D, fatty acids including PUFAs ^g . |
| 41 (1) | α -lactalbumin-1 | gi 301783679 | 8(4) | 115 | Regulatory subunit of lactose synthase. Changes the substrate specificity of galactosyltransferase making glucose a good acceptor substrate for this enzyme, and enabling LS to synthesize lactose. |
| 42 (1) | Whey acidic protein | gi 301777338 | 23(14) | 422 | Function unclear. Possibly associated with innate immunity; possible role in regulation of mammary epithelial cell proliferation. |
| 43 (1) | β -lactoglobulin-1 | gi 301778533 | 110(33) | 858 | Binds and probably transports retinol (vitamin A), vitamin D, fatty acids including PUFAs ^g . |
| 44 (1) | β -casein | gi 301768260 | 85(32) | 815 | Phosphoprotein. Source of amino acids, delivers calcium, phosphate, lipids. Determines the surface properties of the casein micelles. |
| 45 (1) | Haptoglobin | gi 301776456 | 105(43) | 926 | Indicator of infection or inflammation. Acute phase protein. Captures free haemoglobin. Anti-microbial. Also found to high confidence by 2-D gel analysis in 12 hours post-partum sample from the same animal. |

^a Gel bands as indicated in Figure S1.

^b identification

^c NCBI Genbank GenInfo accession codes.

^d Number of peptides found to match with number of peptides unique to this identification in parentheses.

^e MASCOT (MOWSE) search score where scores greater than 38 are taken to be significant.

^f Putative functions as listed in NCBI GenBank and UniProtKB databases.

^g Highly abundant in many mammalian milks. Some species produce only one isoform, and others, such as humans, produce none. PUFAs, polyunsaturated fatty acids.

These identifications were confirmed from 2-D gels carried out on Li Li's milk sampled 12 hours after birth and on day 22.

gi|xxx Accession number from NCBI Genbank database for *Canis lupus familiaris genome* database.

*This identification from BLAST searching of GenBank with nearest similarity in *Canis lupus familiaris* database, but also *Ursus maritimus*; the *A. melanoleuca* NCBI GenBank GeneInfo accession code given here.

Table S3. Proteins in giant panda milk. Identification of the proteins isolated from bands excised from the 1-D SDS-PAGE gels shown in Figure 3 of the main paper, and as an expansion of table 1 of the main paper. The putative functions and comments are drawn from a variety of sources including NCBI and UniProtKB/Swiss-Prot databases.

| Band label ^a | Protein identification ^b | Accession number ^c | Number of matches (unique peptides) ^d | Mascot score ^e | Putative function and comments ^f |
|-------------------------|--------------------------------------|-------------------------------|--|---------------------------|---|
| A | Immunoglobulin λ light chain | gi 281339129* | 1(1) | 78 | Light chain isoform associated with all immunoglobulin subclasses. |
| | Immunoglobulin μ heavy chain | gi 62183982 | 2(0) | 63 | Heavy chain of immunoglobulin M (IgM). Antibody. Abundant in serum, less so in secretions. Abundant in colostrum.** |
| B | Immunoglobulin α | gi 62183972 | 20(5) | 167 | Heavy chain of immunoglobulin A (IgA). Antibody. Abundant in secretions (e.g. tears, saliva, bile, milks). |
| C | Immunoglobulin α | gi 62183972 | 45(14) | 379 | Heavy chain constant region of immunoglobulin A (IgA). Antibody. Abundant in secretions (e.g. tears, saliva, bile, milks). |
| | Polymeric immunoglobulin receptor | gi 301767800 | 23(9) | 290 | Receptor for IgA and IgM mediating secretion, part of which (secretory piece) binds to IgA to protect it against proteolytic cleavage in intestine. |
| D | Immunoglobulin γ heavy chain | gi 62183980 | 39(12) | 430 | Heavy chain of immunoglobulin G (IgG). Antibody. Abundant in serum, less so in secretions. |
| E | Immunoglobulin γ heavy chain | gi 62183980 | 15(6) | 201 | Heavy chain of immunoglobulin G (IgG). Antibody. Abundant in serum, less so in secretions. |
| F | Bile salt-activated lipase *** | gi 301777998 | 22(11) | 238 | Presumed to assist with digestion of lipids, triglycerides in particular. Also termed bile salt stimulated- or dependent-lipase. |
| G | β -lactoglobulin-2 | gi 301778529 | 2(1) | 70 | Bind and probably transport retinol (vitamin A), vitamin D, fatty acids including PUFAs ^g . |
| | β -lactoglobulin-1 | gi 281347108 | 7(2) | 69 | |
| | κ -casein | gi 281347661 | 3(1) | 46 | Stabilizes micelle formation, preventing casein precipitation in milk. |
| H | β -casein | gi 301768260 | 39(13) | 362 | Source of amino acids, delivers calcium, phosphate, lipids. |

| Band label ^a | Protein identification ^b | Accession number ^c | Number of matches (unique peptides) ^d | Mascot score ^e | Putative function and comments ^f |
|-------------------------|-------------------------------------|-------------------------------|--|---------------------------|---|
| I | Lactotransferrin | gi 301754041 | 1(1) | 48 | Iron binding transport proteins with anti-bacterial properties. Low quality identification. |
| J | β -lactoglobulin-1 | gi 301778533 | 9(2) | 81 | Binds and probably transports retinol (vitamin A), vitamin D, fatty acids including PUFAs ^g . |
| K | β -lactoglobulin isoform 1 | gi 301778529 | 9(3) | 75 | Binds and probably transports retinol (vitamin A), vitamin D, fatty acids including PUFAs ^g . |
| | Anti-leukoproteinase | gi 301778531 | 6(3) | 74 | Acid-stable proteinase inhibitor with strong affinities for trypsin, chymotrypsin, elastase, and cathepsin G. |
| L | Lysozyme C | gi 301783675 | 14(6) | 265 | Milk isoform of lysozyme. Anti-bacterial. |
| M | Whey acidic protein | gi 301777338 | 4(2) | 81 | Function unclear. Possibly associated with innate immunity. Possibly plays role in regulation of the proliferation of mammary epithelial cells. |
| | Lysozyme C | gi 301783675 | 1(1) | 55 | Milk isoform of lysozyme. Anti-bacterial. |
| N | Xanthine dehydrogenase | gi 301783299 | 42(19) | 528 | Milk fat globule secretion. Innate immunity. |
| O | Bile salt-activated lipase *** | gi 301777998 | 22(4) | 158 | Presumed to assist with digestion of lipids, triglycerides in particular. Also termed bile salt stimulated- or dependent-lipase. |
| P | Polymeric immunoglobulin receptor | gi 301767800 | 16(7) | 217 | Receptor for IgA and IgM mediating secretion, part of which (secretory piece) binds to IgA to protect it against proteolytic cleavage in intestine. |
| Q | Immunoglobulin γ heavy chain | gi 62183980 | 20(7) | 222 | Immunoglobulin G (IgG). Antibody. Abundant in serum, less so in secretions. |
| R | β -casein | gi 301768260 | 5(0) | 53 | Phosphoprotein. Source of amino acids, delivers calcium, phosphate, lipids. Determines the surface properties of the casein micelles. |

| Band label^a | Protein identification^b | Accession number^c | Number of matches (unique peptides)^d | Mascot score^e | Putative function and comments^f |
|-------------------------------|---|-------------------------------------|--|---------------------------------|--|
| S | Apolipoprotein D isoform 2 | gi 301772170 | 14(6) | 163 | Lipid transporter. |
| | β -casein | gi 301768260 | 8(2) | 62 | Source of amino acids, delivers calcium, phosphate, lipids. |
| | Immunoglobulin λ light chain | gi 73995681* | 5(1) | 60 | Light chain isoform associated with all immunoglobulin subclasses. |
| T | Immunoglobulin λ light chain | gi 281339129* | 41(14) | 467 | Immunoglobulin G (IgG). Antibody. Abundant in serum, less so in secretions. One of two light-chain isoforms associated with all immunoglobulin subclasses. |
| U | β -lactoglobulin-1 | gi 301778533 | 7(3) | 64 | Binds and probably transports retinol (vitamin A), vitamin D, fatty acids including PUFAs ^g . |

^a Gel bands as indicated in Figure 3.

^b Protein identification

^c NCBI Genbank accession codes and MASCOT data (number of unique peptides, sequence coverage, MASCOT score).

^d Number of peptides found to match with number of peptides unique to this identification in parenthesis.

^e MASCOT (MOWSE) search score where scores greater than 38 are taken to be significant.

^f Putative functions as listed in NCBI GenBank and UniProtKB databases.

^g PUFAs, polyunsaturated fatty acids. Highly abundant in many mammalian milks. Some species produce only one isoform, and others, such as humans, produce none.

*This identification from BLAST searching of GenBank with nearest similarity in *Canis lupus familiaris* database, but also *Ursus maritimus*; the *A. melanoleuca* NCBI GenBank GeneInfo accession code given here.

**Good identification as an immunoglobulin. Low level but positive identification as containing immunoglobulin μ chain, but migration rate in protein electrophoresis consistent with IgM.

*** One of the pandas (Xiao Yatou) produced a higher molecular sized isoform of this enzyme, possibly reflecting a polymorphism in glycosylation of this enzyme within the species (not shown). Such glycosylation could be relevant to protection against pathogens (ref. (1) and see also below).

For this table, all known identifications of trypsin (autoproteolysis during trypsin digestion stage) and keratin peaks (from panda or human skin and hair) were excluded.

Table S4. Relative abundance of the 10 most common small molecules in giant panda milks in colostrum and mature milk, compared with mature phase human and cow milks. Relative abundance is ranked according to peak heights and areas under the mass spectrometry data peaks. The giant panda samples were collected on the indicated days postpartum and the human and bovine samples were collected during mid lactation. Compounds listed in bold are discussed in the text. Time-dependent trends of fucosyllactose and lactose are presented in Figures 4 and S3, respectively. PC, phosphatidylcholine. PE, phosphatidylethanolamine.

| Giant panda day 3 | Giant panda day 46 | Giant panda day 158 | Human | Cow |
|----------------------------|---------------------------|----------------------------|---------------------|------------------------|
| | | | | |
| Glycerophosphocholine | Glycerophosphocholine | Glycerophosphocholine | Lactose | Valine |
| Sialyl lactose | Sialyl lactose | Fucosyllactose | Choline | Creatine |
| Lactose | Lactose | Creatinine | Glutamine | acetylcarnitine |
| Creatine | Lyso PC 16:0 | Carnitine | Alanine | Lactose |
| Lyso PC 16:0 | Carnitine | Lyso PC 16:0 | Acetylcarnitine | Creatinine |
| Creatinine | Taurine | Glycerophosphoethanolamine | Leucine | N-acetylglucosamine |
| Lyso PE 18:1 | Lyso PE 18:1 | Lyso PE 18:1 | Glutamate | Carnitine |
| Taurine | Acetyl carnitine | Taurine | Glucose | riboflavin |
| Glycerophosphoethanolamine | Pentanoylcarnitine | Lyso PE 18:1 | N-acetylglucosamine | N-acetylanthranilate |
| Lyso PC 18:0 | Choline | Formiminotetrahydrofolate | Stachyose | Propionyl choline |
| N-acetylgalactosamine | Formiminotetrahydrofolate | Choline | Threonine | Octadecadienylglycerol |

Table S5. Milk oligosaccharides reported in this and previous studies on giant pandas and other species of bear, and their post-partum trends in giant panda milk. This list is confined to those oligosaccharides definitively identified in this study – others were found but not fully identified. Their details are available on request. See also Figure S3 for graphical presentations of trends in concentrations with time after birth.

| Chemical formula | Subunit formula and name of compound | Reference | Observed post-partum trend in giant panda milk (this study) |
|----------------------------------|---|------------------|--|
| Found in giant panda milk | | | |
| C12H22O11 | Lactose * | (2, 3) | Initially high, gradually decreases. |
| C23H39NO19 | Neu5Ac(α 2-3)Gal(β 1-4)Glc (3'-N-acetylneuraminylactose) | (2, 3) | Initially high, gradually decreases. |
| C23H39NO19 | Neu5Ac(α 2-3)Gal(β 1-4)Glc (6'-N-acetylneuraminylactose) | (2) | Initially low, gradual increase to plateau after 20 days. |
| C29H49NO23 | Neu5Ac(α 2-3)Gal(β 1-4)Fuc(α 1-3)Glc | (2) | Zero or low, rapid increase after 7 days to plateau after 20 days. |
| C18H32O16 | Gal(α 1-3)Gal(β 1-4)Glc (isoglobotriose) | (2, 3) | Gradual increase to plateau after 20 days. |
| C24H42O20 | Gal(α 1-3)Gal(β 1-4)Fuc(α 1-3)Glc (fucosyl isoglobotriose) | (2, 3) | Initially zero or low then rapid increase after 7 days to plateau after 20 days. |

* Lactose is the dominant sugar in cow and human milks, but is low or absent in the milks of many Carnivora.

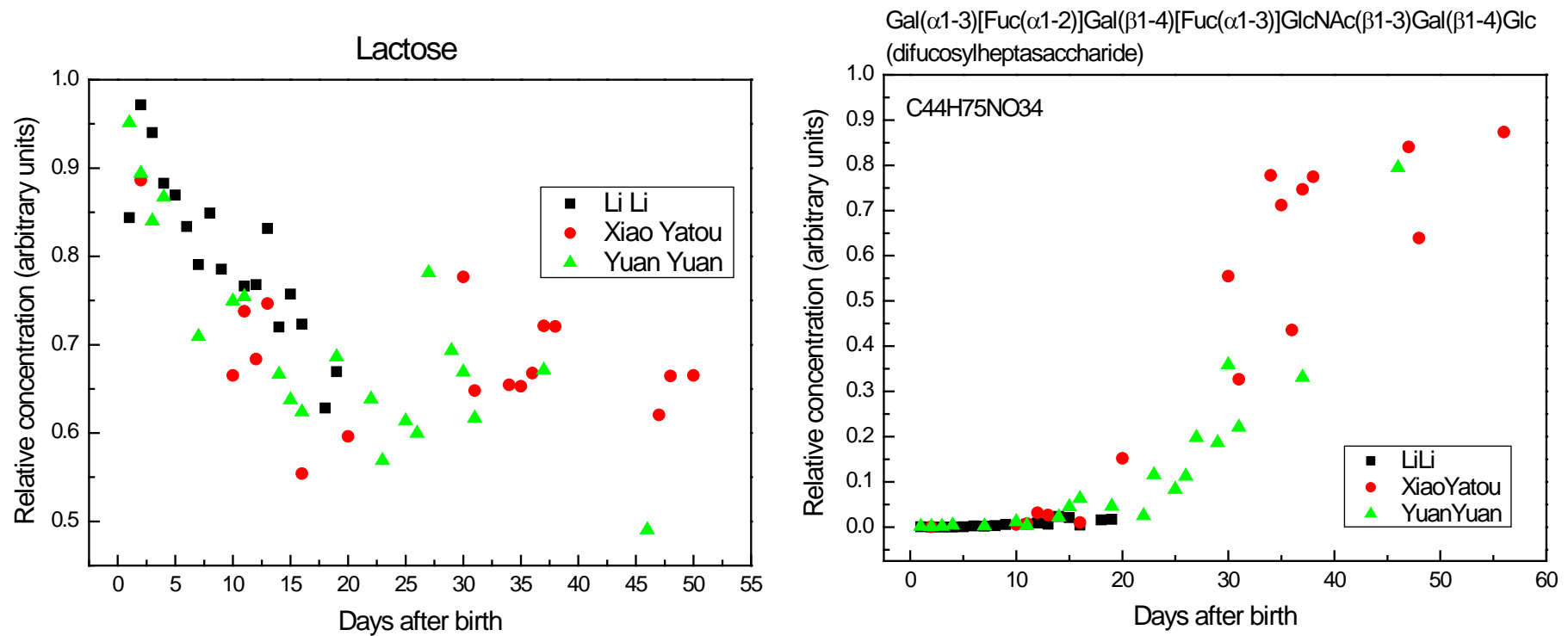
Table S5 continued.

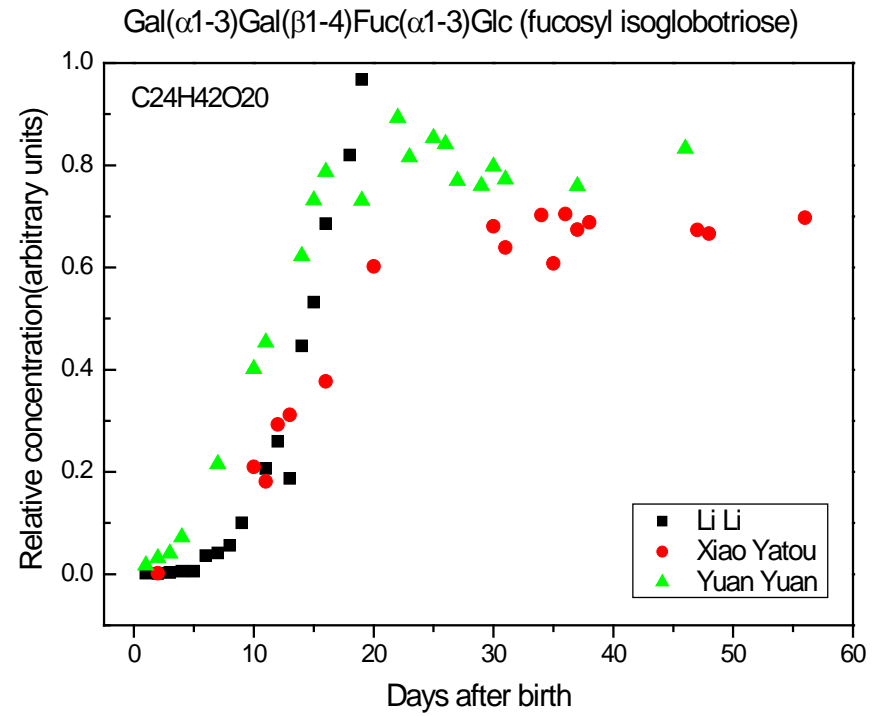
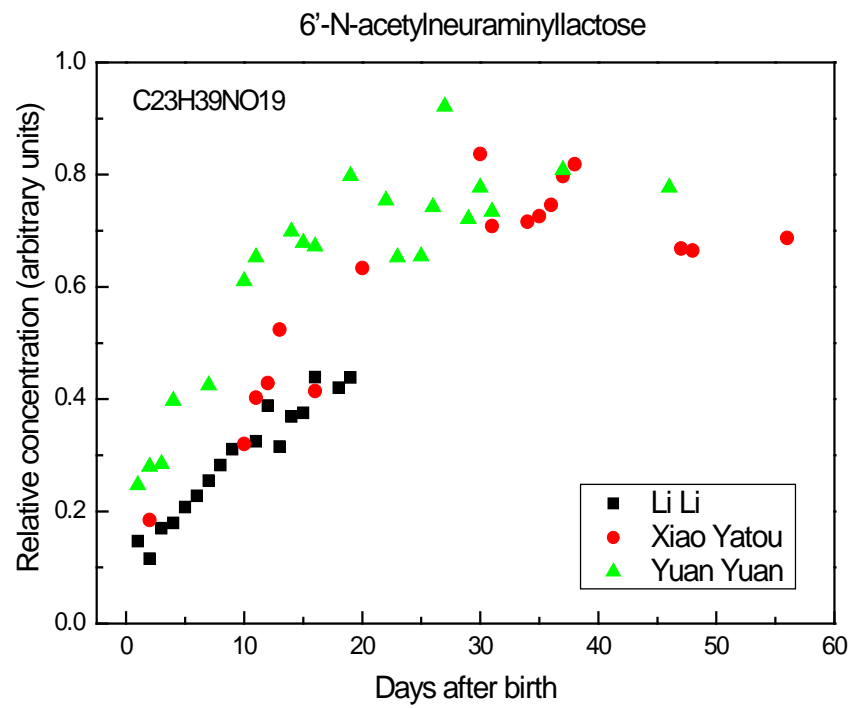
| Chemical formula | Subunit formula and name of compound | Reference | Observed post-partum trend in giant panda milk (this study) |
|---|---|------------------|--|
| Found in both giant panda milk and in the milks of other species of bear | | | |
| C18H32O15 | Fuc(α 1-2)Gal(β 1-4)Glc (2'-fucosyl lactose) | (3-6) | Initially low then dramatic increase after 20 days. |

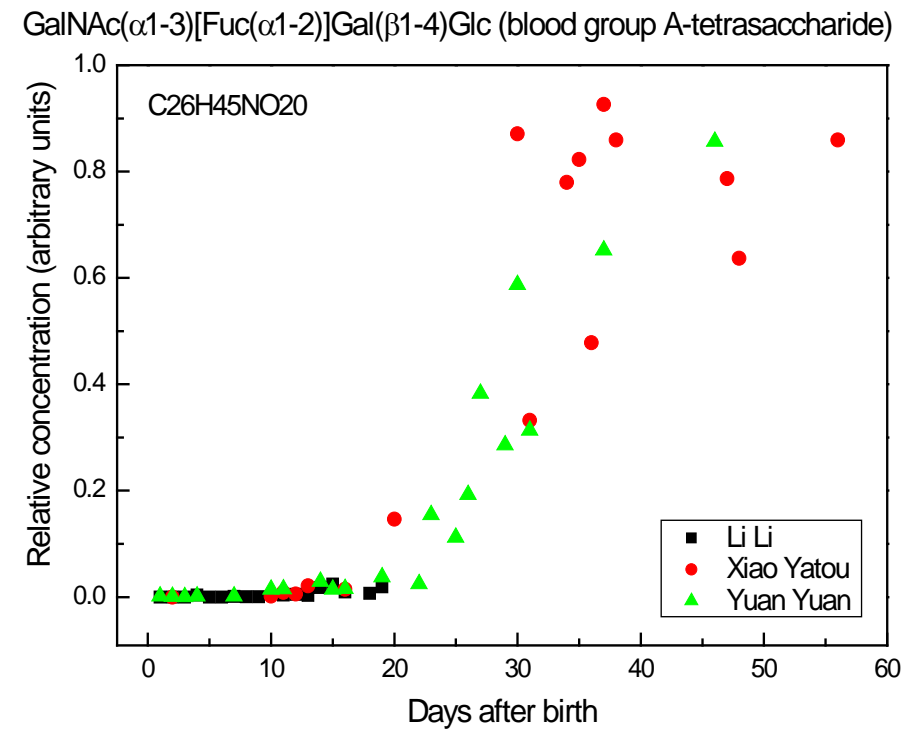
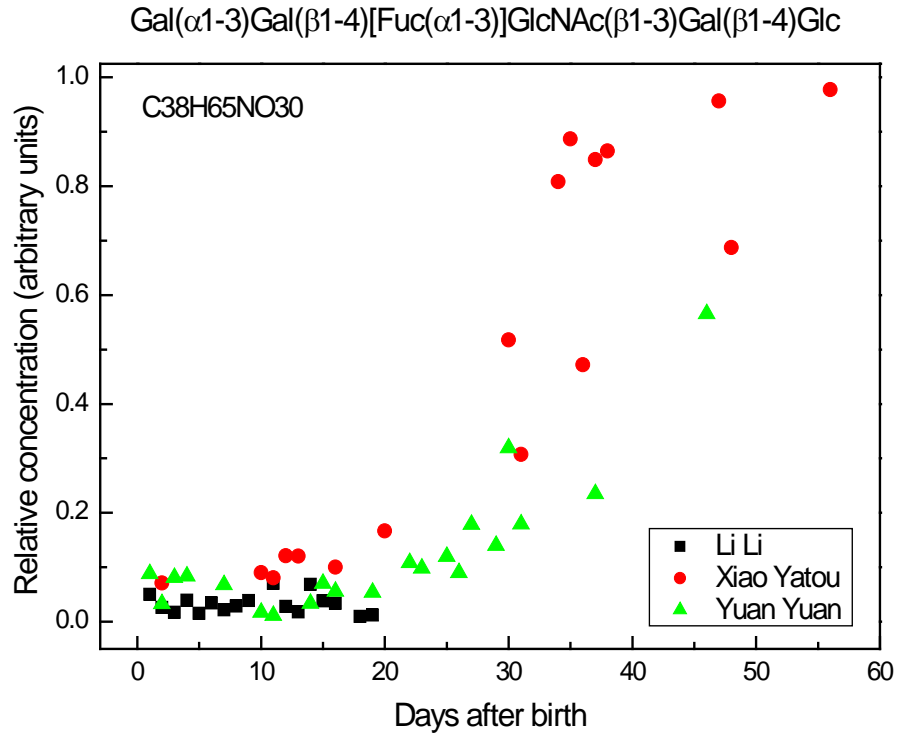
| | | | |
|--------------|--|--------|---|
| C26H45NO20 | GalNAc(α 1-3)[Fuc(α 1-2)]Gal(β 1-4)Glc (tetrasaccharide) (human blood group A determinant) | (3, 6) | Zero or low until 20 days then dramatic increase. |
| C62H102N4O47 | Neu5Ac(α 2-6)Gal(β 1-4)GlcNAc(β 1-3)[Neu5Ac(α 2-6)Gal(β 1-4)GlcNAc(β 1-6)]Gal(β 1-4)Glc | (7) | Initially low then increase after 20 days. |
| C38H65NO30 | Gal(α 1-3)Gal(β 1-4)[Fuc(α 1-3)]GlcNAc(β 1-3)Gal(β 1-4)Glc | (4, 6) | Low until 20 days then rapid increase. |
| C44H75NO34 | Gal(α 1-3)[Fuc(α 1-2)]Gal(β 1-4)[Fuc(α 1-3)]GlcNAc(β 1-3)Gal(β 1-4)Glc (difucosylheptasaccharide) | (5) | Low until 20 days then rapid increase. |
| C63H105N3O48 | Neu5Ac(α 2-6)Gal(β 1-4)GlcNAc(β 1-3){Gal(α 1-3)Gal(β 1-4)[Fuc(α 1-3)]GlcNAc(β 1-6)}Gal(β 1-4)Glc | (7) | Initially low then increases after 20 days. |
| C64H108N2O49 | Gal(α 1-3)Gal(β 1-4)[Fuc(α 1-3)]GlcNAc(β 1-3)[Gal(α 1-3)Gal(β 1-4)[Fuc(α 1-3)]GlcNAc(β 1-6)]Gal(β 1-4)Glc (difucosyldecasaccharide) | (5, 6) | Initially low then increases after 20 days. |

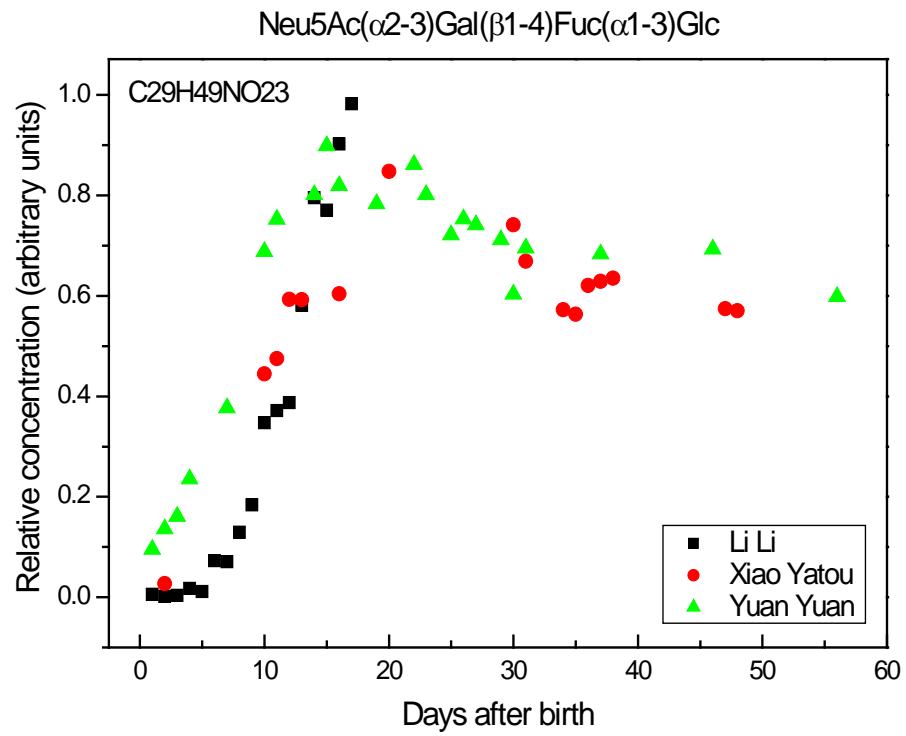
Figure S3. Diversity in the concentration time courses of selected giant panda milk oligosaccharides with time after birth.

Time course plots of seven of the oligosaccharides listed in table S4 to illustrate the diversity in the timings of their appearances or reductions with time after birth. Particularly notable are those that are undetectable at first then rise in relative concentrations abruptly but at different times.









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