



The Role of the Microbiome and Allergic Disease in Premature Infants

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Outline

- Background on microbiome and the association with atopy
- Study design
- Preliminary findings
- Future directions

Association Between Microbiome and Atopic Disease

- Asthma: most common chronic disease in pediatrics
 - Greater risk in pre-term
- Food allergy: early exposure recommended
 - Prevalence appears to be rising $\sim 1.2\%$ /decade
- Microbiome:
 - Hygiene hypothesis - increased susceptibility to allergic disease with lack of early childhood exposure to infection, symbiotic micro-organisms and parasites
 - Lower rates of allergic disease:
 - Antibiotics
 - Exclusive breastfeeding for first 4 months
 - Vaginal delivery
 - Furry pets

Microbiome Components

- Microbiome: sum of microbes, genomic elements and interactions in a given ecological niche
 - 10^6 microbe genes vs 2300 human genes
- Definitions
 - Probiotic: live microbes
 - Prebiotic: non-digestible compounds (HMOs)
 - Synbiotic: pro- + prebiotic
- Symbiosis:
 - Modulate type II immunity
 - Intestinal barrier function
 - Mucosal immune system

Dysbiosis – Affects on Food Allergy and Occurrence in Prematurity

- Microbiome alterations: antibiotics, diet, infections, bacterial exposure
- Dysbiosis
 - Change in food allergy outcomes
 - Timing – first 100 days of life ¹
- Prematurity:
 - Development in the hospital
 - Multiple insults
 - Microbiome \sim # NICU days ²

1. Arrieta, M.C. (2015). Early infancy microbial and metabolic alterations affect risk of childhood asthma. *Sci Transl Med*, 7(307): 1-14

2. Neu, J. (2015). Preterm infant nutrition, gut bacteria and necrotizing enterocolitis. *Curr Opin Clin Nutr*, 18(3): 285-88.

The Association Between Milk Feedings in the Preterm Population, the Microbiome and Risk of Atopic Disease

- Prospective cohort study
- Hypotheses:
 - Specific microbiome pattern will be affected by nutrition, antibiotics and acid suppressive meds
 - Microbiome patterns will be associated with the development of atopic disease
 - Correlate maternal diet with presence of breast milk peptides
- Inclusion: ≤ 34 WGA, BW ≤ 2000 g in NICU
- Exclusion: major congenital abnormalities on GI tract or precluding trial completion, HIE

Procedures	NICU	NICU Discharge	4 months	12 months	24 months	4-6 years
Consent	•					
Medical History	•	•	•	•	•	•
Gestational Age	•	•				
Adjusted Age	•	•	•	•	•	•
Growth Parameters	•	•	•	•	•	•
Demographics	•	•	•	•	•	•
Stool Sample	•	•	•	•	•	•
Milk Sample	•	•	•	•		
Oral Sample	•	•	•	•	⏏	⏏
ISAAC Questionnaires			•	•	•	•
ImmunoCAP Multiple Allergen			•	•	•	
History and Allergy Questionnaire	•					
Visit Questionnaire			•	•	•	•

Milk Analysis

- Mass Spectrometry
- University of Nebraska protein database

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NP_000468.1      MKWVTFISLLFLFSSAYSRGVFRRDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPF
NP_851335.1      MKWVTFISLLLLLFSSAYSRGVFRRDTHKSEIAHRFKDLGEEHFKGLVLIAFSQYLQQCPF
NP_001005208.1   MKWVTFISLLFLFSSAYSRGVFRRDTYKSEIAHRFKDLGEQYFKGLVLIAFSQHLQQCPY
                  *****:*****:***:*****: **.*****:*****:
                  :
NP_000468.1      EDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEP
NP_851335.1      DEHVKLVNELTEFAKTCVADESHAGCEKSLHTLFGDELCKVASLRETYGDMADCCCKQEP
NP_001005208.1   EEHVKLVREVTEFAKTCVADESAENCDKSIHTLFGDKLCAIPSLREHYGDLADCCEKEEP
                  :*****.*:***** .*:***:*****:*. :.*** **:***** :**
                  :
NP_000468.1      ERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLF
NP_851335.1      ERNECFLSHKDDSPDLPKL-KPDPNTLCDEFKADEKKFWGKYLYEIARRHPYFYAPELLY
NP_001005208.1   ERNECFLQHKNDNPDI PKL-KPDPVALCADFQED EQKFWGKYLYEIARRHPYFYAPELLY
                  *****.*:*.*:*.*.*: .*: .:* *: :*:.* *****:
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Protein Group	Protein ID	Accession	-10lgP	Coverage (%)	Coverage (%) Sample 2	Area Sample 2	#Peptides	#Unique	#Spec Sample 2	PTM	Avg. Mass	Description
1	3	P02788 TRFL_HUMAN	442.15	84	84	5.99E10	223	216	7478	Y	78182	Lactotransferrin OS=Homo sapiens GN=LTF PE=1 SV=6
9	7	P49327 FAS_HUMAN	392.45	63	63	1.73E9	125	124	1023	Y	273424	Fatty acid synthase OS=Homo sapiens GN=FASN PE=1 SV=3
5	4	P47989 XDH_HUMAN	378.34	66	66	3.26E9	104	103	1251	Y	146424	Xanthine dehydrogenase/oxidase OS=Homo sapiens GN=XDH PE=1 SV=4
3	24	P05814 CASB_HUMAN	364.22	88	88	7.79E10	199	199	2983	Y	25382	Beta-casein OS=Homo sapiens GN=CSN2 PE=1 SV=4
4	5	P01833 PIGR_HUMAN	354.99	64	64	4.91E9	89	89	1280	Y	83284	Polymeric immunoglobulin receptor OS=Homo sapiens GN=PIGR PE=1 SV=4
16	6	P19835 CEL_HUMAN	345.13	52	52	2.56E9	68	68	726	Y	79322	Bile salt-activated lipase OS=Homo sapiens GN=CEL PE=1 SV=3
20	22	P0C0L5 C04B_HUMAN	339.24	47	47	5.39E6	66	3	348	Y	192750	Complement C4-B OS=Homo sapiens GN=C4B PE=1 SV=2
21	23	P0C0L4 C04A_HUMAN	337.17	47	47	3.8E6	66	3	345	Y	192784	Complement C4-A OS=Homo sapiens GN=C4A PE=1 SV=2
7	10	P02768 ALBU_HUMAN	334.76	78	78	3.51E9	82	59	1185	Y	69367	Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2
13	1	P13645 K1C10_HUMAN	334.14	62	62	1.42E9	56	42	796	Y	58827	Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6
27	12	P01024 C03_HUMAN	332.73	47	47	1.52E8	62	61	205	Y	187147	Complement C3 OS=Homo sapiens GN=C3 PE=1 SV=2
12	11	P04264 K2C1_HUMAN	330.94	67	67	1.31E9	62	52	773	Y	66039	Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6
22	13	P22897 MRC1_HUMAN	327.94	47	47	3.45E8	61	61	349	Y	166011	Macrophage mannose receptor 1 OS=Homo sapiens GN=MRC1 PE=1 SV=1
11	8	Q08431 MFGM_HUMAN	326.16	85	85	2.9E9	52	52	876	Y	43123	Lactadherin OS=Homo sapiens GN=MFG8 PE=1 SV=2
2	101	P00709 LALBA_HUMAN	321.20	80	80	4.41E10	59	58	3251	Y	16225	Alpha-lactalbumin OS=Homo sapiens GN=LALBA PE=1 SV=1
19	9	P35908 K22E_HUMAN	319.10	80	80	5.21E8	57	45	405	Y	65433	Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens GN=KRT2 PE=1 SV=2
14	21	Q13410 BT1A1_HUMAN	313.58	55	55	2.72E9	52	51	825	Y	58960	Butyrophilin subfamily 1 member A1 OS=Homo sapiens GN=BTN1A1 PE=1 SV=3
17	2	P35527 K1C9_HUMAN	313.17	77	77	8.59E8	43	42	548	Y	62064	Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=3
23	28	Q99541 PLIN2_HUMAN	297.97	74	74	9.18E8	37	37	392	Y	48075	Perilipin-2 OS=Homo sapiens GN=PLIN2 PE=1 SV=2
15	33	P01876 IGHA1_HUMAN	289.28	76	76	1.69E9	40	17	726	Y	37655	Ig alpha-1 chain C region OS=Homo sapiens GN=IGHA1 PE=1 SV=2
6	130	P61626 LYSC_HUMAN	289.24	68	68	8.29E9	46	46	1265	Y	16537	Lysozyme C OS=Homo sapiens GN=LYZ PE=1 SV=1
8	68	P47710 CASA1_HUMAN	285.46	65	65	7.34E9	47	47	1121	Y	21671	Alpha-S1-casein OS=Homo sapiens GN=CSN1S1 PE=1 SV=1
10	109	P07498 CASK_HUMAN	283.51	62	62	1.03E10	50	50	905	Y	20305	Kappa-casein OS=Homo sapiens GN=CSN3 PE=1 SV=3
35	19	P02533 K1C14_HUMAN	280.33	66	66	1.72E7	37	2	203	Y	51562	Keratin, type I cytoskeletal 14 OS=Homo sapiens GN=KRT14 PE=1 SV=4
26	14	P48668 K2C6C_HUMAN	280.18	53	53	1.49E6	34	1	192	Y	60025	Keratin, type II cytoskeletal 6C OS=Homo sapiens GN=KRT6C PE=1 SV=3
30	16	P02538 K2C6A_HUMAN	279.00	50	50	2.32E6	33	2	192	Y	60045	Keratin, type II cytoskeletal 6A OS=Homo sapiens GN=KRT6A PE=1 SV=3
28	25	P13647 K2C5_HUMAN	276.95	54	54	5.06E7	34	19	203	Y	62378	Keratin, type II cytoskeletal 5 OS=Homo sapiens GN=KRT5 PE=1 SV=3
34	29	P02787 TRFE_HUMAN	274.93	44	44	1.29E8	29	27	230	Y	77064	Serotransferrin OS=Homo sapiens GN=TF PE=1 SV=3

Milk Protein Alignment

AKH04310.1

[Protein Coverage](#) | [Supporting Peptides](#) |

Protein Coverage:

1 MKLFALLALVLLAGVQSQKDPKLVPDESTLLRLFWEKFDDIADECERFLAPKGYAGVQVSPVHKNLLYTATAPGLWERY
81 QPMSYKLVSRSGDETAFRDMVRRCNAGGVRIYVDVVLNHMSGNWDNAVGTGGSTADTYNYSYPGVDPYDHSDFHPYCILND
161 YQDPEIVRNCELVGLHDLQSQDYVREKLIDFLNHLVDAGVAGFRVDAAKHMWPADLEYIYGKVNNRNSDAGYCGDSSPS
241 RYQEVIDLGGEAVSKFEYNGFGRVTEFKHSEQIGYAFRGNNRLEWITYTKPNWGLLPSGDALVFVDNHDNQRGGGNAILT
321 YKTPKNYKMAIAFILAHPTYGYPRVMSSFDFAHDQGPPQDSDKNILSPSINADGTCGNGWVCEHRWRQHANMVGFRNAVR
401 GTEITNWWDNGNHQIGFCRGDRGFVAFNVEDNDLKQTLQTLCPAGTYCDVISGSKNNRAHLGAELVVAPNGEAFSVRLIS
481 DDDGVLAIHLEEKL

Supporting Peptides:

Peptide	Uniq	-10lgP	Mass	Length	ppm	m/z	z	RT	Fraction	Scan	Source File	Area Sample 2	#Spec	#Spec Sample 2	Start	End	PTM
D.ALVFVDNHDNQR.G	N	57.31	1426.6953	12	5.7	714.3590	2	33.37	3	23088	Milk_1081.raw	2.26E6	3	3	301	312	
L.VFVDNHDNQR.G	N	39.93	1242.5741	10	6.1	622.2981	2	33.33	3	23048	Milk_1081.raw	9.88E4	1	1	303	312	

total 2 peptides

Sample Analyses

- All 4 samples:
 - *Bos taurus* (cow), *Felis catus* (cat), *Canis lupus familiaris* (dog), *Salmo salar* (salmon), *Equus caballus* (horse), *Tyrophagus putrescentiae* (mite/cheese mite), *Aedes aegypti* (mosquito), *D. farinae*, *D. pteronyssinus*, *Hevea brasiliensis* (rubber), *Procambarus clarkii* (crayfish)
- 3/4 samples:
 - *Sus scrofa* (pig), *Cladosporium*, *Gallus gallus* (chicken), *Scylla paramamosain* (crab)
- 2/4 samples:
 - *Oreochromis mossambicus* (tilapia), *Prunus dulcis* (almond), *Crangon crangon* (shrimp), *Triticum aestivum*
- 1/4 samples:
 - *Thunia alba* (orchid), *Periplaneta americana* (cockroach), *Catharanthus roseus* (periwinkle), *Phleum pratense* (timothy grass), *Penicillium citrinum*, *Curvularia lunata* (mold), *Daucus carota* (wild carrot), *Oryza sativa* (rice), *Brassica rapa* (cabbage)

Take-Away Points

- Changes in the microbiome have been associated with different outcomes in asthma, eczema and food allergy
- Premature infants are a special population who are at higher risk for asthma and microbial dysbiosis
- There is a lack of knowledge regarding the role of maternal breast milk in fostering a favorable microbiome profile and its role in allergic sensitization

Future Plans

- Find sequences for all allergenic proteins
- Correlate maternal diet with results from breast milk samples
 - Serial runs on same donor
- Analyze NICU data
- Incorporate HMOs, post-translational modifications into analysis?

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