Methylation, Genomics, and SIBO

Instructor:
Paul Anderson, NMD
Abstract

The world of clinical genomics and nutrigenomics specifically has become an incredibly fast growth and high learning curve area of integrative medicine. Genomics as they affect GI health and SIBO specifically are still in their infancy but are impactful in the pathologies involved and healing from them.

Dr. Anderson will use his knowledge of clinical genomics to propose ways in which ones genes and the epigenetic factors stressing them factor in to SIBO as a pathology and more importantly in healing. A mixture of data, clinical experiences and outcomes and case examples will be used.

Outline

I. Introduction and Presentation of Thesis 20 min / 5 min
   - How genomics relate to GI function and healing
     - Overview of mechanisms
       - Epigenetic effects and SNP triggering
       - Methyl cycle SNPs
       - Immune SNPs
     - Statement of thesis – Nutrigenomics can augment the therapy and healing of a SIBO patient
II. Methyl Cycle Genomics and GI Function 20 min / 15 min
    - How specifically the methyl cycle affects GI health
    - Salient SNP patterns to assess
III. Other Genomics and GI Function 20 min / 15 min
    - Secretor status SNPs
    - LgH related SNPs
    - Other Immunologic SNPs
      - MALT/GALT related SNPs
      - Type 1 and other immune related SNPs
    - Biofilm related SNPs
IV. Therapeutic Inclusion of Nutrigenomics in SIBO Patients 30 min / 15 min
    - Nutrigenomic therapies for Methyl/Cycle SNPs in SIBO
    - Nutrigenomic therapies for Immunologic and Other SNPs in SIBO

Financial Disclosures:

• No relevant financial conflicts to disclose.

• I have no affiliations with any testing company. The use of images depicting particular test reports does not indicate endorsement of said reports.
Introduction:
How Genomics Relate to GI Function and Health

Important Note:
• Many SIBO cases are cleared without the inclusion (knowingly) of any of this information.
• In my experience mindfulness of this information improves outcomes and speed of treatment / strength of recovery.
• I believe genomic factors should be included in all assessments of GI disorders, and especially those cases that do not clear or do not stay cleared.

Overview
• The GI tract has two groups of genomic effectors that crossover its function:
  • Direct: Such as IgA, FUT, Celiac, etc...
  • Indirect: Such as Methylation, Biofilms, Neurotransmission etc...

What tissue specific activities does the methyl cycle have and how do they affect health?
Methylation effects on physiology and health:

• Neurotransmitter (central and peripheral) function:
  • Most neurotransmitters have a methylation step in
    synthesis or degradation:
    • Formation: **DA-NE-Epi-Serotonin**
    • Reduction (partial) **Histamine**

Methylation effects on physiology and health:

• Detoxification
  • Many Phase-2 pathways up-regulate with faster methylation
  • Detoxification of CO2/NH4 [Urea]
  • Biotransformation of some toxins

• Importance in rapidly dividing tissues:
  • Bone marrow function
  • GI repair and maintenance
  • Muscle (skeletal, cardiac and smooth)

Methylation effects on physiology and health:

• DNA Turnover – Cell replication and repair
  • All Purine syntheses and half Pyrimidine syntheses
    is predicated on a methylfolate step
  • No methylation = Very slow or NO DNA repair / replication

**NUCLEOTIDE METABOLISM**

- **PURINE BIOSYNTHESIS**

- **PYRIMIDINE BIOSYNTHESIS**
The possible relationship between the quiescent and the actively cycling nature of the ISCs (Intestinal Stem Cells) needs to be further explored. Moreover, it is critical to understand the genetic elements that determine stem cell fate and the basis by which regeneration occurs in order to better understand stem cell plasticity and the contribution made by the stem cell compartment to malignant disease. It is hoped that future studies in this area will provide a better platform to develop therapies to regenerate damaged intestinal epithelia as seen after radiation injuries or inflammatory bowel disease (eg, Crohn’s disease).

Aside from physiologic and biochemical extrapolations – is anyone researching the genomic – GI connection?
These tight junction components have been shown to affect several signaling and transcriptional pathways, and changes in the expression of tight junction proteins are associated with several disease conditions, such as cancer. Here, we will review how tight junction proteins participate in the regulation of gene expression and cell proliferation, as well as how they are regulated themselves by different mechanisms involved in gene expression and cell differentiation.
The methylation of the gut microbiome: disparate Dam methylation patterns in intestinal Bacteroides dorei

Michael T. Leonard¹, Austin G. Davis-Richardson¹, Alexandria N. Ardissone¹, Kaisa M. Kemppainen¹, Jennifer C. Drew¹, Jorma Ilonen²,³, Mikael Knip⁴,⁵, Olli Simell⁴, Jorma Toppari⁴, Riitta Veijola⁴, Heikki Hyöty⁴ and Eric W. Triplet⁴

...These results suggest that DNA methylation patterns are important to consider in multi-omic analyses of microbiome samples seeking to discover the diversity of bacterial functions and may differ between disease states.

So:

• There is no portion of the GI system which is not affected by genomic influences.
• In some dysfunctional / disease patterns this influence can be life long and create significant dysfunction leading to disease and poor outcome of therapy.
• Assessing and treating nutrigenomic areas in patients such as this can improve outcomes.

Note:

• The rest of the presentation will assume some basic genomic / nutrigenomic understanding.
• Resources will be provided for deeper CME on this topic at the end of the presentation.

A brief review:
SNP

Single Nucleotide Polymorphism - (genetics) genetic variation in a DNA sequence that occurs when a single nucleotide in a genome is altered; SNPs are usually considered to be point mutations that have been evolutionarily successful enough to recur in a significant proportion of the population of a species.

Variations in the DNA sequences of humans can affect how humans develop diseases and respond to pathogens, chemicals, drugs, vaccines, and other agents.

As of 23 July 2013, dbSNP listed 62,676,337 SNPs in humans.

GENETIC - GENOMIC

- Genomics: discipline in genetics that applies recombinant DNA, DNA sequencing methods, and bioinformatics to sequence, assemble, and analyze the function and structure of genomes (the complete set of DNA within a single cell of an organism).

GENETIC - GENOMIC

- Contrast; the investigation of the roles and functions of single genes is a primary focus of molecular biology or genetics and is a common topic of modern medical and biological research. Research of single genes does not fall into the definition of genomics unless the aim of this genetic, pathway, and functional information analysis is to elucidate its effect on, place in, and response to the entire genome's networks.
Note:

- In the following slides the header “Epigenetics” is being used in a very broad sense, including some factors not technically “epigenetic” but fitting the role of factors that affect gene expression, phenotypic outcome and other interactions that can minimize or exaggerate expression of potentially pathogenic SNPs or other phenotypic determinants.

Epigenetics: Patient and family history

- There are “upstream” epigenetic factors that are terribly interesting but we won’t really look at deeply – i.e. experiences of parent or grandparent affect your epigenome...
- Also newer epi or “paragenetic” information (dads stressed sperm affects your DNA etc...)
- But:

EPIGENETIC

“Denoting processes by which heritable modifications in gene function occur without a change in the sequence of the DNA”

Epigenetics is the study of changes in gene expression caused by certain base pairs in DNA, or RNA, being "turned off" or "turned on" again, through chemical reactions. In biology, and specifically genetics, epigenetics is mostly the study of heritable changes that are not caused by changes in the DNA sequence.


EPIGENETIC

To a lesser extent, epigenetics also describes the study of stable, long-term alterations in the transcriptional potential of a cell that are not necessarily heritable. Unlike simple genetics based on changes to the DNA sequence (the genotype), the changes in gene expression or cellular phenotype of epigenetics have other causes, thus use of the term epi- (Greek: επί- over, outside of, around) -genetics. - Specter, Tim (2012). Identically Different: Why You Can Change Your Genes. London: Weidenfeld & Nicolson. p. 8.
The term also refers to the changes themselves: functionally relevant changes to the genome that do not involve a change in the nucleotide sequence. Examples of mechanisms that produce such changes are DNA methylation and histone modification, each of which alters how genes are expressed without altering the underlying DNA sequence.


Further reading for the interested:


So:

• Presence of a SNP does not mean it is activated / expressing

• Epigenetic factors are not “tested” but rather assessed in total

• The more epigenetic stressors the more likely a SNP will be active and expressing

• Many epigenetically activated SNPs can be inactivated / silenced by proper treatment
The Methyl Cycle and GI Function

As mentioned:
Methylation effects on GI physiology and health:

- Affects GI Stem cells, Microbiome, Tight junction function etc...
- DNA Turnover – Cell replication and repair
  - All Purine syntheses and half Pyrimidine syntheses is predicated on a methylfolate step
  - No methylation = Very slow or NO DNA repair / replication

Methyl Cycle – Sulfur AA Interactions (simplified)

What are the components of the methyl cycle and what makes them work?
Quick Methylation Points:

- The whole cycle must be considered and assessed (not just MTHFR).
- In order to normalize (to the degree possible) any methylation imbalances with a nutrigenomic strategy all parts of the methyl cycle must be treated.
- Additionally care should be given in “jump starting” the methyl cycle without assuring collateral - downstream pathways are working first. This avoids the common “detox” and other reactions seen with methylation support applied too quickly.

Where in biochemistry does the methyl cycle exist and what pathways does it intersect with?

Most Common Negative Reactions:

- Negative reaction:
  - Agitation
  - Anxiety
  - Sulfur - Sulfite intolerance
  - Sleeplessness
  - Allergic reactions
  - GI Upset
  - Fatigue
  - Memory problems

- Likely causes:
  - COMT – MAO SNP’s
  - CBS SNP’s
  - Sulfation pathway defects
  - Histamine metabolism
  - Phase-2 overload
Other Genomic / SNP areas
to Consider in
GI Health

Immune System – Genomics

Cell Lines

Bone Marrow:
- Hemocytoblast
- Lymphoid Stem
- Myeloid Stem
- Megakaryocyte
- Proerythroblast
- Monoblast
- Myeloblast
- Platelets
- Retic's

Thymus:
- Lymphoid Stem Cells
- T-Cell Lines

Peripheral Tissues:
- B-Cells
- NK Cells
- Platelets
- Retic's
- PLT
- RBC's
- WBC's

Cell Mediated Immunity
Ab (Humoral) Immunity
Immunological Surveillance

Immune Response

1 – ANTIGEN PRESENTATION
2 – T-CELL ACTIVATION / CMI
3 – B-CELL ACTIVATION / Ab IMMUNITY
3A – ANTIGEN – ANTIBODY REACTION
4 – CLASSIC COMPLEMENT

ATTACK ON INVADER
Antibody – Humoral Response

• **Ig-A**: “Secretory”
  - Primarily the secretory form is the useful kind. (found in tears, saliva, mucus...) Prevents bacteria, viruses, and toxin from attaching to mucosal linings.

• **Ig-E**: “Allergy”
  - Type-1 immediate hypersensitivity (allergy) reactions. Parasite infection.

• **Ig-M**: “First Responder”
  - Elevated in acute infection. Basis for ABO-blood type antigen / transfusion reaction.

• **Ig-G**: “Long Term”
  - Most common type. Focuses NK cells to their targets. Used in passive immunization (gamma-globulin injection).

Compliment

• Activation of endogenous proteins (mainly enzyme precursors) in case of immunologic need.

• Two pathways:
  - **Classical**: activated by antigen – antibody (AG/AB) reaction
  - **Alternate**: goes ‘around’ the AG/AB reaction, activates the compliment cascade in the middle (at the C-3 locus) without the Ag / Ab reaction.
    - Large polysaccharide in the cell membranes of some pathogens stimulate this reaction.
    - Less effective than Classical pathway

Ig issues: Frequency in the chronically ill:

• **Selective Ig-excess**:
  - Ig A excess common in acute and chronic smoldering infections
  - Ig M excess common in acute and chronic smoldering infections
  - IgE Elevation
    - Common in IBD – Parasites / Helminths – significant dysbiosis

• Generally resolve with acute / chronic immune treatment

• **Selective Ig-deficiency**:
  - All potentially can happen and are more common in these cases

• **IgG specific selective deficiency**:
  - Low levels (functional) common
  - Must follow – May respond to IgG Tx.

• **Screening**: [002295] Immunoglobulins A/E/G/M, Serum
  - **IgG Subclasses**: IgG1,2,3,4; Test Number: 209601
Ig: Considerations

Consider these SNP’s in the overall constitution of the patient and their susceptibilities:

- More IgE SNP’s = more Type-1 and Histamine reactions
- More IgA SNP’s = more mucosal issues
- More IgG SNP’s = more immune memory issues

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HLA and Celiac


Abstract

- Celiac disease (CD) is a complex autoimmune disorder caused by ingestion of gluten in genetically susceptible individuals. Finally, we assessed the clinical relevance of this real-time PCR-based assay by studying a cohort of fully characterized patients. As expected, all CD patients had at least one of the CD-associated alleles, and the highest CD risk was indicated by the presence of the HLA-DQ2.5 heterodimer (HLA-DQA1*05-DQB1*02) with HLA-DQB1*02 in homozygosity.
**RAD Complex and Ig**

- Mre11, Rad50, and Nbs1 form an evolutionarily conserved protein complex (Mre11-Rad50-Nbs1, MRN) that has been proposed to function as a DNA damage sensor. Hypomorphic mutations in Mre11 and Nbs1 result in the human...
- Here, we use Cre-loxP-mediated recombination to restrict Nbs1 deletion to B lymphocytes. We find that disruption of Nbs1 results in the accumulation of high levels of spontaneous DNA damage...
- Moreover, we show that Ig class-switch recombination (CSR) is diminished in Nbs1-deficient B cells. The CSR defect is **B cell-intrinsic**, independent of switch-region transcription, and a consequence of inefficient recombination at the DNA level. Our findings reveal that Nbs1 is critical for efficient Ig CSR and maintenance of the integrity of chromosomal structure and number.

PMID: 15668392

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**DARC**

- **INTRODUCTION:** Chemokines are regulated by a family of ‘atypical’ chemokine receptors, D6, DARC and CCX-CKR, each of which efficiently internalizes its cognate chemokine ligands. Development of monoclonal antibodies (MAbs) that would recognize CCX-CKR on the cell surface will be helpful to identify primary CCX-CKR-expressing cell types and analyze the fate of CCX-CKR after ligand binding to the receptor.
- **RESULTS:** A panel of MAbs reacting with CCX-CKR on the cell surface was prepared. The panel was a small one, consisting of only ten MAbs, but was rich in terms of diversity of the Ig isotypes and of the epitopes. Epitope analyses revealed that all the 10 MAbs recognized at least three different, although very close, peptide structures of the N-terminal domain. Three MAbs, namely, 2F11, 13E11 and 14F10, were selected to represent the panel. All of the MAbs were applicable for flow cytometry and immunofluorescent assays and immunoprecipitation. The reactivity of the 2F11 MAb was also confirmed by western blotting. Endogenous expression of CCX-CKR on human hepatocytes and hepatic tumor cell lines was demonstrated using the 13E11 MAb. Interestingly, binding of the 13E11 MAb with B300-19 cells expressing CCX-CKR resulted in induction of CCX-CKR internalization.

PMID: 21184834

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**FCER1**

- IgE has long been known as a therapeutic target for allergic disease, but the difficulty has been in selecting agents that don’t trigger cross linkage of IgE when bound to its high affinity receptor (FceR1) on mast cells and basophils.
- By “designing” a monoclonal antibody (mAb) which targets that part of IgE that binds to that binds to the a-chain of FceR1, the allergic cascade can be effectively interrupted and diseases such as asthma greatly improved, providing a substantial part of their phenotype engages IgE.

Holgate World Allergy Organization Journal 2014, 7:17

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**IL-13**

- IL-13 are able to induce an immunoglobulin isotype switch to IgE in B cells. A major question is to what extent these cytokines contribute to the production of IgE in allergic patients. To address this question we used an in vitro culture system in which the production of IgE is dependent on endogenously produced IL-4 and IL-13.
- Our results indicate that, at least in vitro, IgE production in allergic asthma patients is more dependent on IL-13 than in non-atopics, due to enhanced IL-13 production and to enhanced IgE production in response to IL-13.

FCGR2

- Also known as: CD32; FCG2; FcGR; CD32A; CDw32; FCG2; IGFR2; FCG2A1

- Summary: This gene encodes one member of a family of immunoglobulin Fc receptor genes found on the surface of many immune response cells. The protein encoded by this gene is a cell surface receptor found on phagocytic cells such as macrophages and neutrophils, and is involved in the process of phagocytosis and clearing of immune complexes. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Oct 2008 - NLM]

TNFRSF

- Tumor necrosis factor (TNF) receptor superfamily (TNFRSF), agonistic antibodies have been used to stimulate TNFRSF receptors in vitro and in vivo. TNFRSF receptor-specific antibodies of the IgM subclass and secondary cross-linked or aggregation prone dimeric antibodies typically display superior agonistic activity compared with dimeric antibodies....

- TNFRSF13B/TACI defects have been associated with CVID pathogenesis and/or phenotype, especially the development of benign lymphoproliferation and autoimmunity. Our purpose was to investigate the role ... Both mutations identified in TH patients have been assessed as deleterious for protein function, while the patient with the R202H mutation and sarcoidosis exhibited also sIgG4D. Our study further supports the notion that... but may be also found frequently in distinct clinical phenotypes, including benign lymphoproliferation and IgG subclass deficiencies.

CFH

• We carried out a genome-wide association study of IgA nephropathy, a major cause of kidney failure worldwide. We studied 1,194 cases ... as well as a common deletion of CFHR1 and CFHR3 at chromosome 1q32 and a locus at chromosome 22q12 that each surpassed genome-wide significance.

• These five loci explain 4–7% of the disease variance and up to a tenfold variation in inter individual risk. Many of the alleles that protect against IgA nephropathy impart increased risk for other autoimmune or infectious diseases, and IgA nephropathy

• Nature Genetics 43, 321–327 (2011) doi:10.1038/ng.787

IFIH1

• To understand the genetic predisposition to selective immunoglobulin A deficiency (IgAD), we performed a genome-wide association study in 430 affected individuals (cases) from Sweden and Iceland, and 1,090 ethnically matched controls, and we performed replication studies in two independent European cohorts. In addition to the known association of HLA with IgAD, we identified association with a nonsynonymous variant in IFIH1 (rs1990760G>A, P = 7.3 x 10(-10)) which was previously associated with type 1 diabetes and systemic lupus erythematosus. Variants in CLEC16A, another known autoimmunity locus, showed suggestive evidence for association (rs6498142C>G, P = 1.8 x 10(-7)), and 29 additional loci were identified with P < 5 x 10(-5). A survey in IgAD of 118 validated non-HLA autoimmunity loci indicated a significant enrichment for association with autoimmunity loci as compared to non-autoimmunity loci (P = 9.0 x 10(-4)) or random SNPs across the genome (P < 0.0001). These findings support the hypothesis that autoimmune mechanisms may contribute to the pathogenesis of IgAD.

PMID: 20694011

TRAF1

• This protein and TRAF2 form a heterodimeric complex, which is required for TNF-alpha-mediated activation of MAPK8/JNK and NF-kappaB. The protein complex formed by this protein and TRAF2 also interacts with inhibitor-of-apoptosis proteins (IAPs), and thus mediates the anti-apoptotic signals from TNF receptors. The expression of this protein can be induced by Epstein-Barr virus (EBV). EBV infection membrane protein 1 (LMP1) is found to interact with this and other TRAF proteins; this interaction is thought to link LMP1-mediated B lymphocyte transformation to the signal transduction from TNFR family receptors. [NLM]

• Also related to IgA Nephropathy etc...

HLA-DQA2

• Related to IgA:
  • Nephropathy
  • Deficiency
  • Crosstalk
Ig: Therapy Considerations

Consider these SNP’s in the overall constitution of the patient and their susceptibilities:

• More IgE SNP’s = more Type-1 and Histamine reactions
  • See any of the “Histamine CME” from Dr. Anderson
• More IgA SNP’s = more mucosal issues
  • See below
• More IgG SNP’s = more immune memory issues

Immunoglobulin Stimulation (IgA)

• Colostrum:
  • Crooks CV, Wall CR, Cross ML, Rutherfurd-Markwick KJ. The effect of bovine colostrum supplementation on salivary IgA in distance runners. Int J Sport Nutr Exerc Metab. 2006 Feb;16(1):47-64. PMID:16676703

• Sacro b.:

Immunoglobulin Stimulation (IgG)

• Methylation and Marrow Support
• Mitochondrial Support
• Immune Balancing
Immunogenomics

A BIG topic. I did a webinar for Key2Health you can order and it goes through this topic in a 90 minute webinar format.

“Immunology and genetics: Where to look on the SNP profile for immune affecting genetics and what you can do about them.”


Several autoimmune diseases such as the inflammatory bowel disease (IBD) [9], [21], and type I diabetes [10] have been associated with the FUT2 polymorphism. By studying microbiota of IBD patients with known FUT2 genotypes Rausch et al. showed that the FUT2 genotype could contribute to the susceptibility for IBD through altered microbiota composition [14]. The present study comparing the fecal microbiota in 14 non-secretors and 57 secretors showed that even healthy non-secretors and secretors have differences in their intestinal microbiota. Similarly, differences in composition of mucosal microbiota between healthy non-secretors and secretors were suggested in study by Rausch et al. on the basis of only three healthy non-secretors [14]. Actually, we observed that the bacteria belonging to Blautia et rel., Dorea formicigenaris et rel., Ruminococcus gnarus et rel., and Clostridium sphenoides et rel., were significantly more abundant in the non-secretors than in the secretors. Interestingly, all these taxa have been associated with IBS and/or IBD in several studies [22–24] and may thus indicate that non-secretors have propensity for intestinal aberrations. In addition to IBD, higher susceptibility to e.g. coeliac disease [25] and diabetes type 1 [26] has been associated with the dysbiosis of microbiota and in separate studies also with non-secretor status [10], [27]. Based on these examples it is tempting to speculate that the FUT2 genotype may be a relevant factor that induces alterations in the microbiota composition and plays a role in aetiology of the several diseases involving hostmicrobiota interactions.

This study shows that non-secretors have an altered intestinal microbiota community and strengthens the evidence indicating that the FUT2 polymorphism influence on the intestinal microbiota. The secretor status is one of the drivers of host-associated variation in the microbiota composition and, together with other factors it may contribute to the clustering of microbiota into enterotypes.
Acetaldehyde Exposure

Candida

Another important exposure route to toxic acetaldehyde levels is through its production by the opportunistic yeast, Candida albicans. In small numbers, this yeast may be kept in check in the gut by the immune system and friendly bacteria such as Lactobacillus sp. and Bifidobacterium sp. But in many people, increasing carbohydrates, especially sweets, will cause chronic Candidiasis. Candida produces acetaldehyde in the GI tract by sugar fermentation. The typical American diet along with drug and antibiotic therapies, hypochlorhydria (low stomach acid), chronic stress, environmental toxins, etc. have altered gut integrity and immunity and predisposed millions of people to yeast overgrowth or the “Candida Syndrome.”4 A person with this condition who also drinks beer, wine or liqueurs not only produces acetaldehyde from the alcohol but also delivers more sugar for yeast production of acetaldehyde, creating a double-barreled dose. Acetaldehyde produced in the gut can eventually reach more parts of the body, flooding the system and increasing the risk for damage.5

Acetaldehyde Exposure

Pollution

Through the burning of tobacco, petroleum fuels, natural gas, wood and trash, aldehydes, including acetaldehyde, are present in the air we breathe. Vehicle and factory exhaust can create a chronic but significant exposure source to those who live near heavily trafficked areas or who spend hours commuting on freeways. Acetaldehyde contributes to photochemical “smog” formation when it reacts with other volatile substances in the air. Open car windows increase exposure, as does breathing in acetaldehyde-containing fumes near gas pumps. Cigarette smokers and others around them are exposed through inhaling smoke. According to the Environmental Protection Agency (EPA), wood smoke from campfires, wood-burning stoves and residential fireplaces is more toxic than cigarette smoke. But the acetaldehyde level released from burning items such as plastics, styrofoam and batteries is even higher.6 While acetaldehyde exposure from auto exhaust and cigarettes may be less than that from alcohol, research shows that low dose chronic exposure may still be sufficient to gradually damage proteins, enzymes and other cellular structures in the brain and other organs.7

Furthermore, most fragrances today are made from synthetic chemicals, many of which are toxic. Air fresheners, scented candles, cleaning products, cologne or perfume and more can create a source of chronic exposure to many toxic chemicals including acetaldehyde. Children and babies are particularly susceptible. Additionally, the Environmental Working Group (EWG) lists acetaldehyde as one of the contaminants released from polyethylene plastic bottles.8

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Alcohol dehydrogenases (ADH) (EC 1.1.1.1) are a group of dehydrogenase enzymes that occur in many organisms and facilitate the interconversion between alcohols and aldehydes or ketones with the reduction of nicotinamide adenine dinucleotide (NAD+ to NADH). In humans and many other animals, they serve to break down alcohols that otherwise are toxic, and they also participate in generation of useful aldehyde, ketone, or alcohol groups during biosynthesis of various metabolites. In yeast, plants, and many bacteria, some alcohol dehydrogenases catalyze the opposite reaction as part of fermentation to ensure a constant supply of NAD+.
Acetaldehyde and Nutrient Deficiencies

In addition to its toxic effects, acetaldehyde induces deficiencies of nutrients used for its detoxification. As an example, vitamin B1 (thiamine) is depleted through alcohol and acetaldehyde detoxification. B1 is essential in carbohydrate metabolism for energy production, of which the brain uses 20 percent. **Acetaldehyde-induced B1 depletions** exacerbate the already low B1 levels common in the population due to diuretics and other drugs, over-consumption of simple carbohydrates (diabetes) and adrenal stress. In addition to its many functions, thiamine, the “nerve vitamin,” is critical to nerves and neurotransmitters. Even mild, chronic B1 deficiency can produce brain-related symptoms such as emotional instability, confusion, depression, fatigue, irritability, headaches, sensitivity to noise, insomnia, decreased short-term memory, brain-fog and a feeling of impending doom.17-18

Relevant to this time of year, B1 deficiency-related lactic acidosis can make people more vulnerable to bug bites, since many insects, particularly mosquitoes, are attracted to mild acids.19 Furthermore, people with chemical sensitivities to aldehydes may also be sensitive to seemingly unrelated substances like sulfites (preservatives) from wines and foods, and the smell of chlorine from pools and bleach.


**ACETALDEHYDE METABOLISM AND DETOXIFICATION**

- **ALD2**
  - ALD2 plays a crucial role in maintaining low blood levels of acetaldehyde during alcohol oxidation. In this pathway, the intermediate structures can be toxic, and health problems arise when those intermediates cannot be cleared.[3] When high levels of acetaldehyde occur in the blood, facial flushing, light headedness, palpitations, nausea, and general “hangover” symptoms occur. These symptoms are indicative of a disease known as the Alcohol flush reaction, also known as “Asian Flush” or “Oriental Flushing Syndrome”. [6]


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ALD2

Aldehyde dehydrogenase is a polymorphic enzyme\[3\] responsible for the oxidation of aldehydes to carboxylic acids, which leave the liver and are metabolized by the body's muscle and heart.\[3\] There are three different classes of these enzymes in mammals: class 1 (low Km, cytosolic), class 2 (low Km, mitochondrial), and class 3 (high Km, such as those expressed in tumors, stomach, and cornea). In all three classes, constitutive and inducible forms exist. ALDH1 and ALDH2 are the most important enzymes for aldehyde oxidation, and both are tetrameric enzymes composed of 54kDA subunits. But are at the highest concentration in the liver.\[3\]


http://jn.nutrition.org/content/128/2/459S/F1.expansion

CYP’s (Phase-1) in the GI Tract


OBJECTIVE

Although the human small intestine serves primarily as an absorptive organ for nutrients and water, it also has the ability to metabolise drugs. Interest in the small intestine as a drug-metabolising organ has been increasing since the realisation that it is probably the most important extrahepatic site of drug biotransformation.

KEY FINDINGS:

Among the metabolising enzymes present in the small intestinal mucosa, the cytochromes P450 (CYPs) are of particular importance, being responsible for the majority of phase I drug metabolism reactions. Many drug interactions involving induction or inhibition of CYP enzymes, in particular CYP3A, have been proposed to occur substantially at the level of the intestine rather than exclusively within the liver, as originally thought. CYP3A and CYP2D represent the major intestinal CYPs, accounting for approximately 80% and 18%, respectively, of total immunonquantitated CYPs. CYP2J2 is also consistently expressed in the human gut wall. In the case of CYP1A1, large interindividual variation in the expression levels has been reported. Data for the intestinal expression of the other human CYPs is conflicting. Several CYPs, including the common hepatic isoform CYP2E1, are also expressed to a very low extent, if at all. The distribution of most CYP enzymes is not uniform along the human gastrointestinal tract, being generally higher in the proximal regions of the small intestine.

SUMMARY:

This article reviews the current state of knowledge of CYP enzyme expression in human small intestine, the role of the gut wall in CYP-mediated metabolism, and how this metabolism limits the bioavailability of orally administered drugs. Possible interactions between drugs and CYP activity in the small intestine are also discussed.
Mitochondria

• While we have spoken about mitochondria in the original (Key2Health and CCNM) series on genomics, and in many other settings such as the immune and fatigue oriented CME on the ConsultDrA website, it is critical to remember that mitochondria need support in chronic GI disease.
• The longer the dysregulation the more the mitochondria get “run down”.
• In an immunologically challenged person if they have a number of mito-SNP’s their resistance, bone marrow function and recovery will be significantly affected.

Mitochondrial Damage

• Big topic – Involved in the pathology of most chronic – slow resolution cases
• Slow to repair, but as it does the major symptoms lessen or are eliminated
• Concepts are:
  • Clean up (curcumin)
  • Restore energy (Poly-MVA, B-Vitamins, Iron [ferritin over 30-40 minimum], Thyroid etc.)
  • Repair (Phospholipids, ALA, Carnitine, Taurine)
  • Attend to cell ReDox: Omegas / Tocopherols / Ascorbate / Glutathione

Mitochondrial Damage

• Big topic – Involved in the pathology of most chronic ID folks
• Slow to repair, but as it does the major symptoms lessen or are eliminated
• Again a whole 90 min course is available:
  https://www.consultdranderson.com/product/mitochondrial-function/

Cofactors to consider:

• Nicotinamide / NADH
• Co-Q-10
• Riboflavin-5-phosphate
• Iron
• Proline
• Ca, Mg, K, Zn, Cu, Cr [positive]
• Cd [negative]

•Structure 19, 833–843, June 8, 2011
Biofilms:
They make tests falsely negative, slow healing and cause the “recurrent” cases we see all the time.

Some (of the many) resources:

- npj Biofilms and Microbiomes (2015) 1, 15005; doi:10.1038/npjbiofilms.2015.5; published online 25 March 2015

Biofilm Agents – A Spectrum:

1. Prevention:
   A. Inhibit: Quorum Sensing
      I. Organism cell signaling with auto-inducers which determines gene expression, virulence, resistance, and the development of biofilms.
   B. Inhibit: Initial Attachment of Biofilm Colonies
   C. Inhibit: Organism Efflux Pump / Multi Drug Resistance Pump Inhibitors

2. Active therapies:
   A. Bacteriostatic & 'cidal agents
   B. Direct biofilm disruption agents

Oral Biofilm Rx: Results

Phase-1 (attachment / early biofilm)   Phase-2 Later Biofilm

- As compared to preventive phase-1 agents?
- As compared to other phase-2 agents?

BOTTOM LINE: YOU CANNOT TREAT PHASE-2 BIOFILMS (which all your chronically ill folks have) WITH PHASE-1 THERAPIES.
Biofilm Agents – A Spectrum: 1-Prevention

- Enzymes
- Aromatics
- Tannins
- Phenolics
- Xylitol, Stevia
- Black cumin
- Etc...

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Biofilm Agents – A Spectrum: 2- Active Therapy

- Antimicrobial Therapies
  - Natural (including Black Cumin)
  - Synthetic
- Direct Biofilm Disruption
  - Agents that actually disrupt and “open” the biofilm

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Oral Biofilm – Bismuth-Thiol Complex

- STRONGEST PHASE-2 AGENT AVAILABLE:
- More than the sum of its parts
- Pharmacology very different from individual parts

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Oral Biofilm Rx: FAQ

- Isn’t bismuth toxic?
  - Not in this form. This is neither bismuth nor thiol. The reason a reactive form of bismuth and thiol(s) are mixed is to create a NEW molecule. The new molecule is what disrupts the biofilm.

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Oral Biofilm Rx: FAQ

• Won’t it chelate my patient?
  • Same answer – no. The Dithiol is bound to the bismuth so the toxicity of bismuth and chelating ability of the thiols are negated.

Biofilm Rx: FAQ

• In all your research and human trial is IV administered biofilm therapy more likely to “stir up” or aggravate a patient or is oral biofilm Rx more likely?
  • In almost every case we have seen MUCH more aggravation in oral biofilm therapy. Far less with IV therapies.
  • This is likely due to the fact that biofilms are thought to start in the GI tract.

Oral Biofilm Rx: FAQ

• Does the initiation of immune symptoms after starting the agent mean it is not working?
  • No. In fact it means it is working. You may need more anti-infective, endocrine, inflammatory or other support as the symptoms mean the immune system may be “seeing” the ID agents for the first time (due to opening of the biofilm).

Oral Biofilm Rx:

• Any **compounding pharmacy** can make these capsules based on the strongest ingredients available in the studies mentioned.
  • Initial testing on humans shows very good tolerance.
• **Formula:**
  • DMPS 25mg/ Alpha Lipoic Acid 100mg/ Bismuth **Subnitrate** 200mg per Capsule
  • Ideally no substitutions
  • DMSA 100 mg can sub for DMPS
  • Bismuth **Subcitrate** can sub for Subnitrate (will make product weaker)
Oral Biofilm Rx:

• Bis-thiol dosing:
  • Approximates the most potent – most researched biofilm drugs
  • Uses the most (available) forms and combinations of medication chemistry
• DOSE:
  • 1 cap QD away from food, 3X a week for one week as a test dose
  • 1-4 caps QD to BiD away from food 3-5X a week
  • Extra doses (on the “off days”) are helpful in dermatologic flares during therapy for dermatologic and ‘Herx’ type reactions
  • Once an immunologic reaction is reached the dose may need to be decreased as needed

Normal trial is for 60 – 120 days during other anti-infective therapy

• May be used much longer if clinically indicated

Usual trajectory of therapy:

• First 30-60 days may have no change
• Eventually (when the biofilm Rx breaks the biofilm open) the patient will typically exhibit signs of an immune reaction. This can be any cytokine based reaction.
  • This is the time when a balance must be struck:

Biofilm Rx Support:

• This “balance is gained typically by allowing the biofilm Rx to continue and modulating anti-infective Rx along with enough Adrenal (and occasionally Thyroid) support.
• If the patient is on non-Rx adrenal support they may need 5-10X the dose for a time.
• If they are on low dose hydrocortisone and adrenal support they often will need more hydrocortisone (sometimes 2-4X for a time).

What is next?

Phase-1 (attachment / early biofilm)  Phase-2 Later Biofilm

• After you have broken through the biofilm and therapy is progressing (which may take 3-12 months) then you can phase in the “phase-1” agents to clean up the biofilms that are most clinically significant – AND – keep them from re-forming.
Biofilms

• While we do not have the time to delve as deeply as necessary into this topic I have a full 90 minute webinar which can be obtained at the URL below.
• It develops the concepts of phases of biofilms and how those phases affect treatment (and why most ‘biofilm therapies’ do not work.)
• Additionally specific recommendations are given for each phase of biofilm activity and natural as well as Compounded Rx agents available.

https://www.consultdranderson.com/product/biofilms-update/

Important Note:

• Many SIBO cases are cleared without the inclusion (knowingly) of any of this information.
• In my experience mindfulness of this information improves outcomes and speed of treatment / strength of recovery.
• I believe genomic factors should be included in all assessments of GI disorders, and especially those cases that do not clear or do not stay cleared.

“A.L.T.O.S.”

• ASSESS Sn/Sx and SNP’s
• Start LOW and work up
• TREAT the patient not just the SNP’s
• Treat from the OUTSIDE in
• Monitor SIGNS AND SYMPTOMS to adjust therapy
**Concept:**

- A genetic slow-down or absence of an enzyme system will cause:
  - Slower production of the enzyme, nucleotide, neurotransmitter etc
  - Slower elimination of the used product
  - Imbalance in products
  - Excess consumption of collateral (or possibly primary) pathway cofactors
  - All of the above

**Treatment Order: Outside - In**

- Diet – GI [within other therapeutic limitations]
  - Colorful veggies / flavinoids
  - Avoid sensitivities and sugar
  - Long term of course **but you can’t out supplement a shitty diet**
- Inflammation and Phase-2 Support
  - Most “SNP” support eventually hits some oxidative pathway or another
  - Many post SNP Tx reactions are Phase 1 → 2 back ups etc

**Treatment Order: Outside - In**

- Trace Minerals + Magnesium + MVM if tolerated
  - Almost all patients are trace mineral deficient
  - NO B-vitamin Tx can work without its collateral trace element
  - Always consider Taurine in Magnesium sensitive people
  - Also K+ in Magnesium sensitive people
- Non Methyl Donor additions – as indicated(B1,2,3,5,6)
  - See dosing below
- Methyl Support – as indicated
  - See dosing below
  - Watch for aggravations:
    - Insomnia / Anxiety / Agitation
    - Pain / Detox-like Sx...

---

Nutrient dosing over time:

Dose:
- High [Pharmacologic]
- Repletion
- Test Dose

Time

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Nutrient dosing over time:

Dose:
- High [Pharmacologic]
- Acute Dosing
- Replete / Pharmacologic Dosing
- Maintenance / Balance Dosing

Time

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Nutrient dosing over time: B-1 Example

Dose:
- High [Pharmacologic]
- Acute Dosing
- Replete / Pharmacologic Dosing
- Maintenance / Balance Dosing

- "Test dose"
- "Replete and compensate"
- "Maintain"

Time

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Nutrient dosing over time: B-2 Example

Dose:
- High [Pharmacologic]
- Acute Dosing
- Replete / Pharmacologic Dosing
- Maintenance / Balance Dosing

- "Test dose"
- "Replete and compensate"
- "Maintain"

Time

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Nutrient dosing over time: B-3 Example
Expressed as Niacinamide dose form:

<table>
<thead>
<tr>
<th>Dose:</th>
<th>High [Pharmacologic]</th>
<th>Repletion</th>
<th>Test Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>Dosing 100 - 500 mg</td>
<td>Replete / Pharmacologic</td>
<td>Maintenance / Balance Dosing 50 – 500 mg dep. on SNP's</td>
</tr>
<tr>
<td>Test Dose</td>
<td>&quot;Test dose&quot;</td>
<td>&quot;Replete and compensate&quot;</td>
<td>&quot;Maintain&quot;</td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(c) PS Anderson - www.ConsultDrA.com 2016

Nutrient dosing over time: B-5 Example

<table>
<thead>
<tr>
<th>Dose:</th>
<th>High [Pharmacologic]</th>
<th>Repletion</th>
<th>Test Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>Dosing 250 mg</td>
<td>Replete / Pharmacologic</td>
<td>Maintenance / Balance Dosing 100 - 250</td>
</tr>
<tr>
<td>Test Dose</td>
<td>&quot;Test dose&quot;</td>
<td>&quot;Replete and compensate&quot;</td>
<td>&quot;Maintain&quot;</td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(c) PS Anderson - www.ConsultDrA.com 2016

Nutrient dosing over time: P-5-P Example

<table>
<thead>
<tr>
<th>Dose:</th>
<th>High [Pharmacologic]</th>
<th>Repletion</th>
<th>Test Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>Dosing 10 - 50 mg</td>
<td>Replete / Pharmacologic</td>
<td>Maintenance / Balance Dosing 15 - 50 dep. on SNP's</td>
</tr>
<tr>
<td>Test Dose</td>
<td>&quot;Test dose&quot;</td>
<td>&quot;Replete and compensate&quot;</td>
<td>&quot;Maintain&quot;</td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(c) PS Anderson - www.ConsultDrA.com 2016

Nutrient dosing over time: 5-MTHF / Folinic Acid Example

<table>
<thead>
<tr>
<th>Dose:</th>
<th>High [Pharmacologic]</th>
<th>Repletion</th>
<th>Test Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>Dosing 200 - 400 mcg</td>
<td>Replete / Pharmacologic</td>
<td>Maintenance / Balance Dosing 400 mcg – 5 mg dep. on SNP's</td>
</tr>
<tr>
<td>Test Dose</td>
<td>&quot;Test dose&quot;</td>
<td>&quot;Replete and compensate&quot;</td>
<td>&quot;Maintain&quot;</td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Forms of Folate:**

- **Folic Acid** requires DHF reducetase
- **Folinic Acid / Leukovorin** (formyl-THF) No DHF reducetase required
- **5-Methyltetrahydrofolate**

(Graphics) [http://web2.iadfw.net/uthman/nutritional_anemia/nutritional_anemia.html](http://web2.iadfw.net/uthman/nutritional_anemia/nutritional_anemia.html)

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**Nutrient dosing over time: B-12 Example**

Methyl – Hydroxy or Adeno forms

<table>
<thead>
<tr>
<th>Dose:</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
</tr>
<tr>
<td>[Pharmacologic]</td>
</tr>
</tbody>
</table>

- **Repletion**
  - Acute Dosing 200 - 400 mcg
  - Replete / Pharmacologic Dosing 1 to 10 mg
  - Maintenance / Balance Dosing 1 – 5 mg dep. on SNP’s

- **Test Dose**
  - “Test dose”
  - “Replete and compensate”
  - “Maintain”

---

**Nutrient dosing over time: Vitamin-C Example**

Given in divided doses

<table>
<thead>
<tr>
<th>Dose:</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
</tr>
<tr>
<td>[Pharmacologic]</td>
</tr>
</tbody>
</table>

- **Repletion**
  - Acute Dosing 500 – 1000 mg
  - Replete / Pharmacologic Dosing Bowel Tolerance
  - Maintenance / Balance Dosing 1 - 2 grams

- **Test Dose**
  - “Test dose”
  - “Replete and compensate”
  - “Maintain”

---

**Nutrient dosing over time: Magnesium Example**

Citrate or Glycinate form – Divided Doses

<table>
<thead>
<tr>
<th>Dose:</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
</tr>
<tr>
<td>[Pharmacologic]</td>
</tr>
</tbody>
</table>

- **Repletion**
  - Acute Dosing 100 – 200 mg
  - Replete / Pharmacologic Dosing Bowel Tolerance
  - Maintenance / Balance Dosing 150 – 400 mg

- **Test Dose**
  - “Test dose”
  - “Replete and compensate”
  - “Maintain”
Nutrient dosing over time: Copper Example

Dose:
- High [Pharmacologic]

Repletion
- Acute Dosing 1-3 mg
- Replete / Pharmacologic Dosing 2-6 mg
- Maintenance / Balance Dosing 1-3 mg

Test Dose
- "Test dose"
- "Replete and compensate"
- "Maintain"

Time

Nutrient dosing over time: Taurine Example

Dose:
- High [Pharmacologic]

Repletion
- Acute Dosing 250-500 mg
- Replete / Pharmacologic Dosing 1000-4000 mg
- Maintenance / Balance Dosing 500-1000 mg

Test Dose
- "Test dose"
- "Replete and compensate"
- "Maintain"

Time

VITAMIN | GENERAL ENZYME SUPPORT FUNCTION
---|---
B-1 | ALDEHYDE transfer / DECARBOXYLATION
B-2 | H+ Transfer / FMN – FAD (Flavins)
B-3 | H+ Transfer / NAD – NADP
B-5 | ACYL Group Transfer / Co-A
B-6 | AMINO Group transfer / De- & Trans “aminations” [DAO, GAD, GABA]
BH4 a.k.a. (tetrahydrobipterin) | NOS Cofactor / Superoxide anion producer; SAMe Cofactor in Hydroxylase reactions
SAMe | COMT, 5HT AND TYR Hydroxylase Support
ASC | H+ Transfer / Hydroxylation of Proline& Lysine
GSH | Glutathione Transferase
BIOTIN | CARBOXYLATION

Cofactors: Inorganic / Metal Ions

<table>
<thead>
<tr>
<th>Ion</th>
<th>Examples of enzymes containing this ion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cupric</td>
<td>Cytochrome oxidase</td>
</tr>
<tr>
<td>Ferrous or Ferric</td>
<td>Catalase / Cytochrome (via Heme) / Nitrogenase / Hydrogenase</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Glucose 6-phosphatase / Hexokinase / DNA polymerase</td>
</tr>
<tr>
<td>Manganese</td>
<td>Arginase</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>Sulfite Oxidase / Nitrate reductase / Nitrogenase</td>
</tr>
<tr>
<td>Nickel</td>
<td>Urease</td>
</tr>
<tr>
<td>Zinc</td>
<td>Alcohol dehydrogenase / Carbonic anhydrase / DNA polymerase</td>
</tr>
</tbody>
</table>
Remember:

• If your patient is healthy and you are providing preventive assessment and treatment your therapeutic role is neither this complicated nor aggressive.
• Assure proper diet with precursors
• Support globally based on MTHFR status, family history, age etc...
• Since you do not know how toxic or how many SNP’s they have – Be as balanced in supplementation as possible and work up.

In non-resolving cases what time frame does it take to have all these therapies effect outcomes?

Online Genomics CME

Key2Health CE Series:
• http://www.keycompounding.com/providers/webinars/introduction-to-clinical-genomics/ [PART-1: SIX CLASSES]

CCNM 6-Part Genomics CME
• http://www.ccnm.edu/nd-careers/continuing-education/ondemand/genomic-medicine-new-applications-clinical-practice

Thanks!
Questions?