Genetic Predisposing Factors in FASD

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Objectives

- Review evidence for genetic influence in FASD
- Review the metabolism of alcohol
- Review results of animal and human gene studies as it relates to alcohol and FASD
- Identify areas of future research priority
- Discuss potential benefits and relative importance of genetic research

How do we know there are genetic influences in FAS?

- Not all fetuses exposed to alcohol are affected
- Animal studies show strain differences
- Varied phenotype observed in FASD may be a reflection of the varied susceptibility quotients in the genetic background of the individual
- High recurrence risk in siblings
- Twin concordance studies (identical > non-identical)
- Ethnic differences in risk for FASD based on epidemiological studies

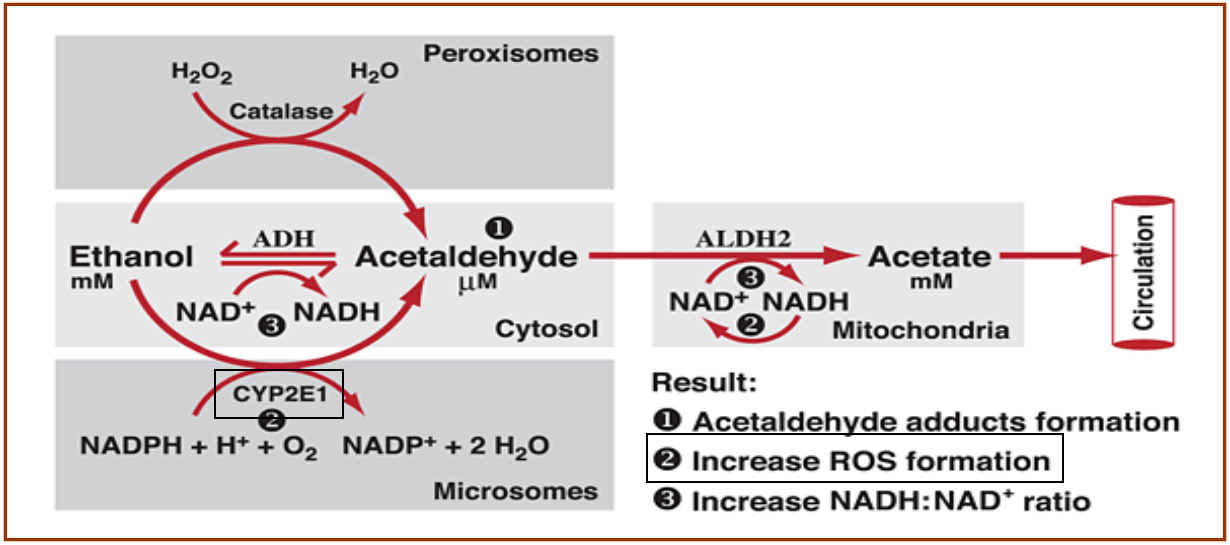
Genetic factors influencing risk of FASD

- Polymorphic variants in enzymes involved in alcohol metabolism (parents and fetal genotypes)
  - Alcoholism, alcohol craving
  - Protection
- Genes involved in brain and organ function and development that are influenced by ethanol exposure (altered promoter region function and gene expression),or through imprinted genes (epigenetic effects)— [↓DNA methylation with ethanol exposure, etc.]

What gene studies have been done in humans?

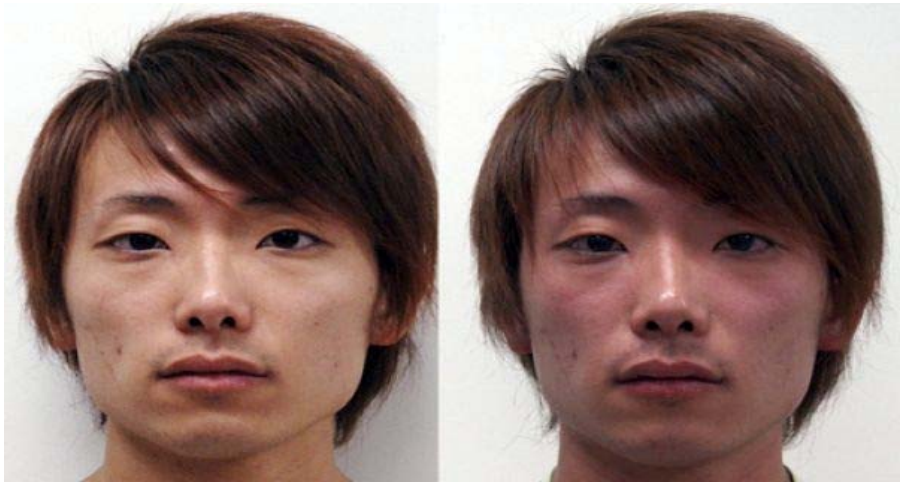
- Case-control studies
  - Need two control groups
    - Non drinking moms and those who drank and had no affected children
- Family linkage analysis studies
  - Few human studies to date
  - Need also paternal DNA
  - Linkage studies have poor success in discovering the genetic causes of complex diseases, because of weak genotype-phenotype association in multi-factorial disorders
- Candidate gene association studies
  - No genome wide or gene expression studies in humans
  - Select genes targeted; mainly those involved in alcohol metabolism, and with conflicting results

Oxidative pathways of alcohol metabolism



http://www.niaaa.nih.gov/Resources/GraphicsGallery/Metabolism/pathways_alcohol.htm

Flushing



People with a relative deficiency of the enzyme ALDH usually don't drink because higher levels of acetaldehyde makes them feel too bad; in addition to flushing they feel nausea and a rapid heartbeat.

Animal studies

Genetic Influence in Alcohol Teratogenesis*

Inbred mouse strain	Treatment (n = live litters)	Malformations			Total percent of litter malformed[a]	Total percent mortality
		Digits	Kidney	Skeletal (vertebral/ribs)		
C57BL/6J	ETOH (20 litters)	19 ($n = 40$)	24 ($n = 34$)	32 (mostly vertebral) ($n = 35$)	44%	11%
	Maltose (19 litters)	0 ($n = 38$)	0 ($n = 35$)	7 (mostly vertebral) ($n = 35$)	4%	11%
DBA/2J	ETOH (17 litters)	0 ($n = 23$)	0 ($n = 19$)	10 ($n = 14$)	19%	41%
	Maltose (19 litters)	0 ($n = 35$)	0 ($n = 21$)	7 ($n = 21$)	7%	21%
A/J	ETOH (16 litters)	0 ($n = 24$)	0 ($n = 21$)	68 (ribs/vertebral) ($n = 19$)	36%	55%
	Maltose (18 litters)	0 ($n = 39$)	0 ($n = 35$)	4 (ribs) ($n = 35$)	9%	36%

Li & Warren, 2005

ADH Family of Genes

- Cluster of 7 genes at 4q with several polymorphisms associated with varied enzyme rates and substrate affinity
- Dimeric protein
- Five classes
- Majority of ethanol is metabolized by isozymes encoded by three members of the ADH class I family: *ADH1A*, *ADH1B*, and *ADH1C*1 and by the class II enzyme encoded by *ADH4*

ADH Family of Genes

- the isozyme encoded by *ADH1B*2* has a turnover rate over 80 times greater than the isozyme encoded by *ADH1B*1*
- the presence of an *ADH1B*2* allele should result in an increase in the rate of ethanol oxidation.

ALDH Genes

- the mitochondrial form of ALDH (ALDH2),as compared with those of the cytosolic form (ALDH1), indicate that it is responsible for oxidizing the majority of the acetaldehyde in the body
- *ALDH2*2* encodes a low activity variant of the mitochondrial ALDH

ADH & ALDH

- both *ADH1B*2* and *ALDH2*2* have been demonstrated to be protective against the development of alcohol dependence

ADH Polymorphisms

Polymorphisms of Alcohol Dehydrogenase (ADH) genes

Class (Protein)	Gene	Subunit	Nucleotide change	Allele frequency
Class I ADH				
ADH1	*ADH1*1*	α		
ADH2	*ADH2*1*	β1		95% of Caucasians 20% of Asians
	*ADH2*2*	β2	47G > A	90% of Asians
	*ADH2*3*	β3	369C > T	24% of Africans
ADH3	*ADH3*1*	γ1		90% of Asians
	*ADH3*2*	γ2	271C > *T*; 349G > A	50% of Caucasians
Class II ADH				
ADH4	*ADH4*	π	192 T > A ; 159G > A; 75A > C	
Class III ADH				
ADH5	*ADH5*	χ		
Class IV ADH				
ADH7	*ADH7*	σ		
Class V ADH				
ADH6	*ADH6*	?[a]		

h.a.: high affinity; l.a.: low affinity.
EtOH: ethanol; RA: retinoic acid.
[a]Subunit composition not known.

Modified from Gemma et al., 2007

ADH Polymorphisms

ADH1B and *ADH1C* Polymorphisms*

Nomenclature		Amino acid differences	Enzyme subunit	Km for EtOH (mM)	Turnover rate (min^{-1})
New	Old				
*ADH1B*1*	*ADH2*1*	Arg47, Arg369	β1	0.05	4
*ADH1B*2*	*ADH2*2*	His47, Arg369	β2	0.9	350
*ADH1B*3*	*ADH2*3*	Arg47, Cys369	β3	40.0	300
*ADH1C*1*	*ADH3*1*	Arg271, Ile349	γ1	1.0	90
*ADH1C*2*	*ADH3*2*	Gln271, Val349	γ2	0.6	40

*Hurley et al., (2002); Human Genome Organization Gene Nomenclature Committee (2001).

Li & Warren *Birth Defects Research (Part A) 73:195–203 (2005)*

Mothers and FAS Children

ADH1B Allele Frequencies in Mixed Ancestry Population from Western Cape Province, South Africa*

			Allele frequencies		
	Number	Allele number	*ADH1B*1*	*ADH1B*2*	*ADH1B*3*
FAS children	56	112	0.928	0.036[a]	0.036
Mothers of FAS children	56	112	0.928	0.036[a]	0.036
Controls	178	356	0.854	0.107	0.039

*Adapted from Viljoen et al. (2001).
[a]Allele frequencies were significantly lower than in controls ($p = 0.025 \pm 0.004$).

Li & Warren, 2005

Ethnic Differences

- Rates FAS higher in African Americans and Native Americans than non-Hispanic whites
- 7 fold higher in Blacks after adjusting for maternal alcohol intake, chronic alcohol problems and age

Ethnic Differences

ADH1B and *ALDH2*: Ethnic Differences*

	*ADH1B*1*	*ADH1B*2*	*ADH1B*3*	*ALDH2*1*	*ALDH2*2*
White (European and American)	>95%	<5%	<5%	100%	0%
Jewish (Israeli and American)	80%	20%	<5%	100%	0%
African American	85%	<5%	22%	100%	0%
Asian (Chinese, Japanese, and Korean)	35%	65%	<5%	70%	30%

*Neumark et al. (1998); Thomasson et al. (1991); Thomasson et al. (1995).

Birth Defects Research (Part A) 73:195–203 (2005)

Li & Warren, 2005

American Indians

Mulligan et al., 2007

- Allelic variants have been identified that alter metabolic rates and influence risk for alcoholism. Specifically, *ADH1B*47His* (previously *ADH2-2*) and *ALDH2-2* have been shown to confer protection against alcoholism
- Protection is presumably through accumulation of acetaldehyde and a resultant 'flushing response' to alcohol consumption.

American Indians

Mulligan et al., 2007

- Both linkage and association analysis identified several *ADH1C* alleles and a neighboring microsatellite marker that affected risk of alcohol dependence and were also related to binge drinking.
- These data strengthen the support for *ADH* as a candidate locus for alcohol dependence and suggest further productive study.

Mothers with FAS children *vs* mothers without FAS children

Observational Study: Free-Choice Drinking and BrAC in a South African Population*

	FAS Mothers (n = 10)		Non-FAS Mothers (n = 20)	
	Mean ± SE	Range	Mean ± SE	Range
Total body weight (kg)	46.2 ± 2.2	33–56	55.9 ± 2.7	42–84
Dose (g of ethanol)	54.3 ± 3.8	40.8–66.0	42.8 ± 1.8	27.2–68.0
Peak breath alcohol concentration [BrAC] (mg%)	125 ± 8	82–161	92 ± 5	51–139
β-60 (mg% per hr)	21.4 ± 1.3	16.4–30.7	20.8 ± 0.8	13.7–28.6
Alcohol elimination rate [AER] (g per hr)	5.5 ± 0.2	4.5–6.6	6.0 ± 0.3	3.8–8.6

*Khaole et al. (2004).

Li & Warren, 2005

“Protective” Polymorphism

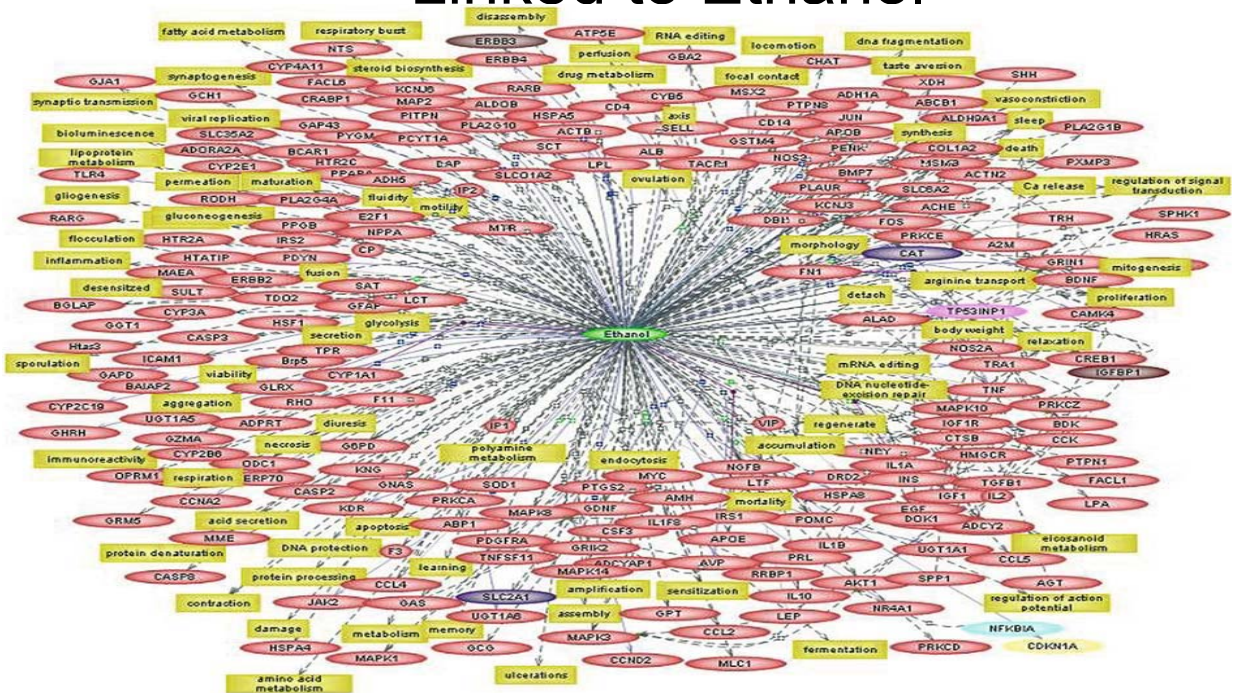
Comparison of Studies on *ADH1B*3* in African Americans

	Detroit[a]	Detroit[b]	Boston[c]
Outcomes examined			
Allele frequency of *ADH1B*3*	[d]	0.20	0.23
Age at assessment	1 year	Up to 7.5 years	Neonate
Birth growth measures	+	+	+
Bayley MDI	+	+	—
Extensive neurodevelopment measures	—	+	—
Facial morphology	+	—	+
Effect of *ADH1B*3* genotype			
Effect of maternal *ADH1B*3*	↓ Risk	↓ Risk	↑ Risk[e]
Effect of fetal *ADH1B*3*	↓ Risk	Not statistically significant	↑ Risk[e]

[a]McCarver et al. (1997) and Das et al. (2004).
[b]Croxford et al. (2003) and Jacobson et al. (2000).
[c]Stoler et al. (2002).
[d]Oversampled.
[e]Independent of alcohol exposure.

Li & Warren, 2005

Genes and Pathways That Are Directly Linked to Ethanol



FROM:

Uddin et al., Neurochem Res, Vol. 30, No. 9, 2005

"Ethanol affects multiple cellular events that include synthesis and degradation, gene regulation, transcription, translation and expression, protein-protein interactions, protein modifications such as phosphorylation, molecular transport and secretion, chromosome assembly, biogenesis and various enzymatic activities.

Therefore, investigating a single or a small number of candidate genes that are involved in ethanol response may not properly identify the actual mechanisms of ethanol action in the brain"..

OR

…or mechanisms causing teratogenic effects (*my extrapolation*).

Uddin et al., Neurochemical Research, Vol. 30, No. 9, 2005

Future Studies

- Convergent Functional Genomics (CFG) is an approach used to identify and prioritize candidate genes, which relies on the cross-matching of animal model gene expression data with human genetic linkage data, as well as human tissue data and biological roles data.

Candidate gene pathways

Table 2: Selected top-ranked candidate genes for FAS identified using binary matrix filtering

Rank	Criteria matched	HGNC ID	Description	Location	Function
1	17/29	*FGFR1*[1]	Fibroblast growth factor receptor 1	8p11.2	Involved in limb induction, play a role in bone elongation modulation
2	16/29	*MSX1*[2]	Msh homeobox homolog 1 gene	4p16.3-p16.1	Potential repressor function in cell cycle progression, transcription repressor
3	15/29	*FGFR2*[1]	Fibroblast growth factor receptor 2	10q26	Involved in vertebral development, important regulator of bone formation and osteoblast activity
4	15/29	*FOXG1B*	Forkhead box G1B	14q13	Embryonic transcriptional regulator, playing a critical role in brain development
5	15/29	*HOXA1*	Homeobox A1	7p15.3	Involved in the placement of hindbrain segments in the proper location along the anterior-posterior axis during development
6	14/29	*BMP4*[2,3]	Bone morphogenetic protein 4	14q22-q23	Regulating myogenesis through dosage-dependent PAX3 expression in pre-myogenic cells, inducing apoptosis and chondrogenesis in the chick limb bud
7	14/29	*FGFR3*[1]	Fibroblast growth factor receptor 3	4p16.3	Negative regulator of bone growth promotion, inhibition of chondrocyte proliferation and differentiation depending on devlopmental time
8	14/29	*GNAS*[2]	Gnas complex locus	20q13.2-q13.3	Involved as modulators or transducers in various transmembrane signaling systems primarily mediating the differential effects of parathyroid hormone
9	14/29	*PAX6*	Paired box gene 6	11p13	Key regulator of eye, pancreas, central nervous system development and regulator of glial precursors in the ventral neural tube

[1] Members of/linked to the MAPK signalling pathway
[2] Members of/linked to the TGF-β signalling pathway
[3]Members of/linked to the Hedgehog signalling pathway

Lombard et al., *BMC Genomics* 2007, **8**:389

Candidate gene pathways

Table 4: Biological pathways significantly over-represented among the top-ranked candidate genes

Pathway	Gene Count	*P*-value[1]	*P*-value[2]
TGF-b signaling pathway	9	0.0000067	0.00001
Hedgehog signaling pathway	7	0.000038	0.000036
MAPK signaling pathway	13	0.000078	0.0006
Adherens junction	7	0.00035	0.00051
Cell cycle	8	0.00036	0.0012
Neurodegenerative disorders	5	0.00075	0.0023
Regulation of actin cytoskeletan	9	0.0035	0.014
Focal adhesion	9	0.004	0.022
Gap junction	6	0.0059	0.0079
Cytokine-cytokine receptor interaction	9	0.011	0.0017
Epithelial cell signaling in H. Pylori infection	4	0.018	0.029

The gene count indicates how many genes from a particular pathway were present in the candidate gene list of 87 genes. Note that varying *P*-values were obtained depending on the background list used
[1]*P*-value obtained using the Homo sapiens gene list as a background list to the top-ranked candidate genes
[2]*P*-value obtained using the original candidate gene list as a background list to the top-ranked candidate genes

Lombard et al., *BMC Genomics* 2007, **8**:389

Candidate gene pathways

- ***TGF-β signalling pathway***
  - These genes play pivotal roles during embryogenesis and development and have a potential role in the distinct characteristics associated with FAS.
  - The pro-apoptotic effect of alcohol provides a probable explanation for the long-term CNS dysfunction and diminished brain size associated with FAS, and could be mediated by the TGF-β pathway.

Candidate gene pathways

- ***MAPK signalling pathway***
  - transmits a large variety of external signals, leading to a wide range of cellular responses, including growth, differentiation, inflammation and apoptosis
  - The MAPK signalling pathway can be activated by external stress factors, such as alcohol
  - experimental manipulation of these second-messenger pathways, through stimulating calcium- and cGMP signalling or inhibiting cAMP signalling, completely reversed the action of ethanol on neuronal migration *in vitro* as well as *in vivo*.

Candidate gene pathways

- ***Hedgehog signalling pathway***
  - This pathway is a key regulator of embryonic development and is highly conserved.
  - FAS animal models portray a strikingly similar craniofacial phenotype to mouse models treated with antibodies that block Hedgehog signalling components, specifically the sonic hedgehog (Shh) molecule
  - alcohol resulted in a significant decrease in Shh levels in the developing embryo, as well as a decrease in the level of other transcripts involved in Shh signalling.
  - the addition of Shh after ethanol treatment led to fewer apoptotic cranial neural crest cells, resulting in a significant decrease in craniofacial anomalies

Epigenetics

Epigenetics refers to changes in phenotype (appearance) or gene expression caused by mechanisms other than changes in the underlying DNA sequence

- DNA methylation and chromatin remodeling/acetylation/phosphorylation
  - Folic acid, homocysteine, choline
- Imprinting
- Ecogenetics

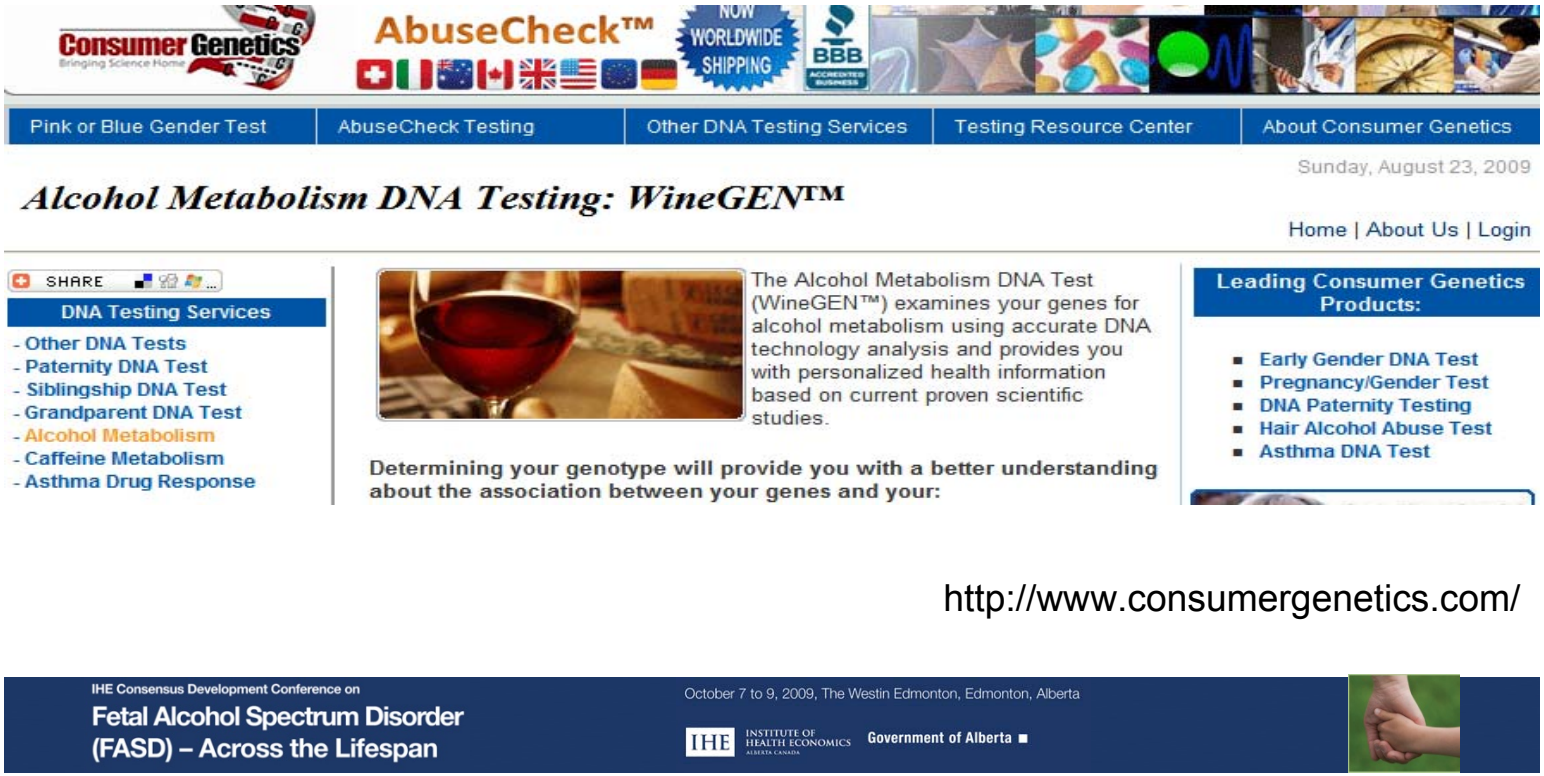
Epigenetics

- Thomas, Abou, Dominguez (2009) demonstrated that choline supplementation reduces effects of prenatal alcohol exposure in rats:
  - Growth, brain size, eye opening, learning and memory
- Choline acts as a methyl donor, influences DNA methylation, is an integral component for cell membrane integrity and is a precursor to the neurotransmitter and neurotophic factor acetylcholine.

Epigenetics

- Hypomethylation of H19 and altered methylation of Igf2 is associated with genetics disorders such as Russell-Silver syndrome and Beckwith-Weidemann syndrome
- Imprinting control in the H19/Igf2 domain may be a mechanism of ethanol-induced growth retardation (Haycock & Ramsay, 2009).

Direct to Consumer DNA Testing



http://www.consumergenetics.com/

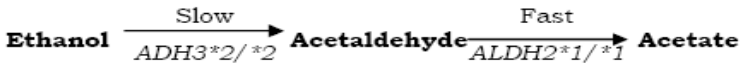
Direct to Consumer DNA Testing

Results:

Alcohol Metabolism:

John Doe is a "SLOW" metabolizer conversion of ethanol to acetaldehyde and a "FAST" metabolizer conversion of acaldehyde to acetate.

Alcohol Dehydrogenase 2 genotype:	ADH2*1/*1 "Slow"
Alcohol Dehydrogenase 3 genotype:	ADH3*2/*2 "Slow"
Aldehyde Dehydrogenase 2 genotype:	ALDH2*1/*1 "Fast"



http://www.consumergenetics.com/

Direct to Consumer DNA Testing

Summary and Interpretations of Results:

Alcohol (ethanol) is carried from the bloodstream to the liver where it is converted into acetate by 2 liver enzymes. The enzyme Alcohol Dehydrogenase (ADH) is responsible for the initial metabolism of alcohol into acetaldehyde, a carcinogen and neurotoxin. Acetaldehyde is then converted into Acetate and Water by the Aldehyde Dehydrogenase the (ALDH) enzyme. Both enzymes are encoded by three genes. Each of these genes can have a "fast" or "slow" version, and when grouped together can determine your acetaldehyde levels and overall metabolism of alcohol.

As a MODERATE DRINKER with a "slow" ADH3*2*2 and "fast" ALDH2*1*1:

- You may benefit from drinking by **decreasing** their risk of heart attack by **31**% [1].
- You may benefit from drinking by **increasing** their high density lipoprotein levels (HDL) by **12**% which lowers cholesterol in your arteries [2].
- You may benefit from drinking by **lowering** their C-reactive protein (CRP) level which in high amounts is associated with tissue inflammation [3].
- You with the "fast" ALDH2*1*1 variant have **no** known risk of alcohol related cancers.

Laboratory specimen was analyzed using DNA isolation and PCR Quantitative Analysis.

http://www.consumergenetics.com/

Conclusions-1

- Although not conclusive, it is probable that allelic variants and other genetic differences influence risk for, and incidence of, FASD, and these risks likely explain differences between ethnic groups.
- Some genetic differences may be protective resulting in less alcohol exposure (flushing effect)
- Certain genotypes may increase drinking (increasing the likelihood for binge drinking and alcoholism) resulting in a higher incidence of FASD.

Conclusions-2

- Further research in genetic and epigenetic factors for FASD may lead to better recognition of at risk individuals and the development of more effective prevention strategies.
- Standardized approaches to determine accurate estimates of maternal alcohol intake and accurate categorize outcomes is essential in future research.

Conclusions-3

- Notwithstanding the enormous biological interest in FASD and importance in understanding mechanisms of alcohol effects, studies identifying genetic risk factors for FASD will not likely have a meaningful impact on the prevalence, treatment or prevention of FASD.