Using Genetic Analysis to Improve Patient Management of Dyslipidemia

Ernst J. Schaefer, Andrew S. Geller, BS, and Margaret R. Diffenderfer, PhD

Cardiovascular Nutrition Laboratory, Human Nutrition Research Center on Aging at Tufts University & Tufts University School of Medicine, Boston, MA, and Boston Heart Diagnostics, Framingham, MA

First Fredrickson Conference, Las Vegas, NV, April 29th, 2018
Donald S. Fredrickson, MD (1924–2002) MD Michigan, medical residency, Brigham, studied cholesterol at MGH, joined NIH in 1953, worked with C B. Anfinsen Jr. (Nobel Prize) and Daniel Steinberg, then set up Molecular Disease Branch, discovered Tangier disease and cholesteryl ester storage disease, was scientific director of the National Heart and Lung Institute and worked with Robert I. Levy, MD and Robert S. Lees, MD to develop new lipoprotein typing system for lipoprotein disorders. ApoA2, C-I, CII, and CIII were isolated and characterized in his branch (the C proteins by Virgil Brown). He also supported Dr. Levy who directed the Lipid Research Clinics Program and Trial. He was director of the Institute of Medicine, then became NIH director, and then director of the Howard Hughes Institute.
W. Virgil Brown, MD (1939-present)
BA Emory ‘60, MD Yale ‘64,
Residency Med. Hopkins ‘66, Clin Assoc. NIH, ’69 where he worked with Donald Fredrickson and Bob Levy to isolate the apoC-II and apoC-III from TG-rich lipoproteins, showing with others that apoC-II activated LPL and that apoC-III inhibited LPL. Endo Yale ’70, then went to UCSD until 1978 where he worked on lipid metabolism and on the LRC Trial, 1978-1987 – Mt Sinai Arteriosclerosis Division, 1987-1991 – Medlantic Research Foundation, Washington DC, 1991-2009 Candler Professor of Med., Emory & Atlanta VA Hospital, founder of NLA and the J Clin. Lipidology, and has made major contributions in research, clinical care, and training of younger clinical researchers.
Boston Heart Diagnostics
Framingham, MA
Conflicts: All authors are employees of Boston Heart Diagnostics (BHD), also all are affiliated with Tufts University.
Definitions of Dyslipidemia

- **Moderate Hypercholesterolemia**: LDL-C > 160 mg/dL*
- **Severe Hypercholesterolemia**: LDL-C > 190 mg/dL*
- **Moderate Hypertriglyceridemia**: TG > 177 mg/dL**
- **Severe Hypertriglyceridemia**: Fasting TG > 885 mg/dL**
- **Moderate HDL Deficiency**: HDL-C < 40 mg/dL in men and < 50 mg/dL in women*
- **Marked HDL deficiency**: HDL-C < 20 mg/dL***
- **Moderate Lp(a) Excess**: Lp(a) > 50 mg/dL****
- **Marked Lp(a) Excess**: Lp(a) > 100 mg/dL
- **Sitosterolemia**: β-sitosterol > 10 mg/L&
- **CTX**: cholestanol > 10 mg/dL&

---

High LDL-C Disorders

- Secondary Hypercholesterolemia
- Familial Hypercholesterolemia
- Familial Combined Hyperlipidemia
- Polygenic Hypercholesterolemia
Secondary Hypercholesterolemia

Secondary causes of elevated LDL-C > 160 mg/dL may include:

- Increased dietary intake of saturated fat and/or trans fats
- Obesity & weight gain
- Hypothyroidism
- Anorexia
- Medications (diuretics, cyclosporin, glucocorticoids, & amiodorone)
- Biliary obstruction
- Nephrotic syndrome

If any of these are present treat appropriately

In a recent study of 1,386 subjects with LDL-C values $> 190$ mg/dL, only 1.7% were noted to have mutations at these three gene loci. Those with a mutation had a 5X higher CVD risk as compared to those that did not (Khera A et al. JACC 2016; 67: 2578-89).

However this analysis may have been flawed because of incomplete informatics on the above three genes, no analysis of other genes causing elevated LDL-C, and no clear assessment of secondary causes of elevated LDL-C.
Secondary Hypercholesterolemia

Of 382,971 subjects (mean age 58, 54% female), 13,966 (3.6%) had LDL-C values ≥ 190 mg/dL (median value 206 mg/dL), and were compared with those having LDL-C < 190 mg/dL (median 108 mg/dL, n=369,005). Significant differences (p<0.0001) below:

- Being female (63.5% vs. 53.6%)
- An Lp(a) value > 50 mg/dL (46% vs. 23%)
- Have the apoE3/4 genotype (29.8% vs. 22.7%)
- Have the apoE4/4 genotype (4.3% vs. 2.2%)
- Elevated TSH (hypothyroidism) (10.5% vs. 7.3%)
- Elevated transaminase value > 120 U/L (0.8% vs. 0.4%)
- No differences in elevated alkaline phosphatase levels
Winners of the 1985 Nobel Prize in Physiology and Medicine “for their discoveries concerning the regulation of cholesterol metabolism.”

Michael S. Brown, MD 1941-present

Joseph L Goldstein, MD 1940-present
Tuboeruptive Xanthomas in FH Homozygote

Tendinous Xanthomas in FH Heterozygote

Arcus Juvenilis in FH Heterozygote

Severe coronary atherosclerosis at autopsy in FH het.
14 year homozygous FH patient at baseline and one year after plasmapheresis every two weeks

Robert A. Hegele, MD, Robarts Research Institute, University of Western Ontario, Canada (Johansen et al J Lipid Res. 2014: 55:765-72)
### Genes for Familial Hypercholesterolemia

- **LDLR** (LDL Receptor)
- **APOB** (Apolipoprotein B)
- **LDLRAP1**
- **PCSK9** (Proprotein Subtilisin Kexin 9)

*Admera (Helix.com), Invitae, and Emory Genetics Laboratory*
<table>
<thead>
<tr>
<th>Genes for Lipid Disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LDLR, APOB, LDLRAP1, PCSK9, ABCG5/8, CYP27A1 (High LDL-C and High Sterols)</strong></td>
</tr>
<tr>
<td><strong>APOA5, APOC2, APOC3, GPIHBP1, LPL, LMF1 (High TG)</strong></td>
</tr>
<tr>
<td><strong>ABCA1, APOA1, LCAT (Low HDL)</strong></td>
</tr>
<tr>
<td><strong>CETP, LIPC, SCARB1 (High Dysfunctional HDL)</strong></td>
</tr>
<tr>
<td><strong>APOE, ANGPTL3, GPD1, LIPA (Combined Hyperlipidemia, Dysbetalipoproteinemia, Cholesteryl Ester Storage Disease or Lysosomal Acid Lipase Deficiency)</strong></td>
</tr>
<tr>
<td><strong>APOB, MTTP, SAR1B (Very Low LDL)</strong></td>
</tr>
</tbody>
</table>

*Emory Genetics Laboratory, Dr. Hegele, London, Ontario*
### Treatment for FH

- Statins
- Ezetimibe
- Colesevelam
- PCSK9 Inhibitors
- Lomitipide
- Mipomersan
- LDL apheresis
William E. Connor, MD (1921–2009)

A pioneer in diet, lipids, and heart disease, MD, residency, research fellowship, & faculty, University of Iowa. In 1974 together with Dr. A Bhattacharyya he was the first to describe 2 sisters with sitosterolemia and tendon xanthomas.

In 1975 he moved to the Oregon Health & Science University where he finished his career (397 publications – 53 years). He established a sterol lab in Oregon (now run by Drs. DeBarber & Duell).

Bill studied the effects of the typical American diet on lipids in the Tarahumara Indians of Mexico, and identified the cause of essential fatty acid deficiency in patients on total parenteral nutrition. He did considerable work on dietary cholesterol & trans fats, but was best known as a pioneer in omega-3 fatty acids - documenting their TG lowering effects.
Sitosteroolemia & FCH

In this population in those with LDL-C > 190 mg/dL a significant differences (p<0.0001) was noted for:

- β-sitosterol value > 99th % (5.4% vs. 1.0%) consistent with phytosteroolemia. In addition 233 (about 1%) had β-sitosterol values > 15.0 mg/L, consistent with homozygous or compound heterozygous sitosteroolemia (ABCG5/8 mutations). Overabsorbers of cholesterol – respond very well to ezetimibe.

- A lathosterol value > than the 99th % (3.6% vs. 1.0%) consistent with familial combined hyperlipidemia, overproducers of cholesterol, respond well to statins.

Dr. Gerald Salen – pioneer in the diagnosis and treatment of cerebrotendinous xanthomatosis (CTX)
Cerebrotendinous Xanthomatosis

- Elevated plasma cholestanol levels > 10 mg/L (Laboratory of Drs. DeBarber & Duell, Oregon Health Sciences University, Portland, OR)
- Patients can present in childhood with chronic diarrhea & learning disabilities
- Can present in adolescence with cataracts
- Can present in their 20s with tendon xanthomas and fairly normal cholesterol levels
- Then can present with neurologic disease.
- Defects in CYP27A1 gene, cannot make chenodeoxycholate (Emory Genetics)
- Treatment: chenodeoxycholate 250 mg po tid

Cerebrotendinous Xanthomatosis

- Elevated plasma cholestanol levels > 10 mg/L (Laboratory of Drs. DeBarber & Duell, Oregon Health Sciences University, Portland, OR)
- Patients can present in childhood with chronic diarrhea & learning disabilities
- Can present in adolescence with cataracts
- Can present in their 20s with tendon xanthomas and fairly normal cholesterol levels
- Then can present with neurologic disease.
- Defects in CYP27A1 gene, cannot make chenodeoxycholate (Emory Genetics)
- Treatment: chenodeoxycholate 250 mg po tid

Cerebrotendinous Xanthomatosis
Cerebrotendinous Xanthomatosis
Definitions of Dyslipidemia

- **Moderate Hypercholesterolemia:** LDL-C > 160 mg/dL*
- **Severe Hypercholesterolemia:** LDL-C > 190 mg/dL*
- **Moderate Hypertriglyceridemia:** TG > 177 mg/dL**
- **Severe Hypertriglyceridemia:** Fasting TG > 885 mg/dL**
- **Moderate HDL Deficiency:** HDL-C < 40 mg/dL in men and < 50 mg/dL in women*
- **Marked HDL deficiency:** HDL-C < 20 mg/dL***
- **Moderate Lp(a) Excess:** Lp(a) > 50 mg/dL****
- **Marked Lp(a) Excess:** Lp(a) > 100 mg/dL
- **Sitosterolemia:** β-sitosterol > 10 mg/L&
- **CTX:** cholestanol > 10 mg/dL&

Diagnosis: Lipoprotein Lipase Deficiency
Defect: Homozygous Amino Acid Substitution

Eruptive Xanthomas

Lipemia Retinalis

Severe Fatty Liver

Pancreatitus with Fat Infiltration

Treatment: Low Fat diet, Abstention From Alcohol, gemfibrozil, 6 grams/day Fish Oil.
Outcome: Stabilization of disease, TG levels leveled off around 700 mg/dl.
# Treatment of Severe Hypertriglyceridemia

- Low Fat, Low Sugar Diet
- Fenofibrate
- Omega-3 Fatty Acids
- Statins
- Combination Therapy
- Anti-ApoC3 Therapy
- Plasmapheresis or LDL apheresis
Definitions of Dyslipidemia

- **Moderate Hypercholesterolemia**: LDL-C > 160 mg/dL*
- **Severe Hypercholesterolemia**: LDL-C > 190 mg/dL*
- **Moderate Hypertriglyceridemia**: TG > 177 mg/dL**
- **Severe Hypertriglyceridemia**: Fasting TG > 885 mg/dL**
- **Moderate HDL Deficiency**: HDL-C < 40 mg/dL in men and < 50 mg/dL in women*
- **Marked HDL deficiency**: HDL-C < 20 mg/dL***
- **Moderate Lp(a) Excess**: Lp(a) > 50 mg/dL****
- **Marked Lp(a) Excess**: Lp(a) > 100 mg/dL
- **Sitosterolemia**: β-sitosterol > 10 mg/L&
- **CTX**: cholestanol > 10 mg/dL&

ApoA-I Deficiency
Tangier Disease
LCAT Deficiency
Fish Eye Disease
Apolipoprotein A-I Immunoblotting in Various Disorders of HDL Metabolism

Diagnosis of Severe HDL Deficiency (HDL-C < 20 mg/dL)

- Measure Lipid Profile and ApoA-I
- ApoA-I Deficiency – normal LDL-C, normal TG, undetectable apoA-I – very premature CVD, APOA1 gene defects
- Tangier Disease (ABCA1 Defects) – low LDL-C, mild TG increase, apoA-I present, but < 10 mg/dL, premature CVD and neurologic disease
- LCAT Deficiency – very low LDL, TG increased, apoA-I usually about 30-40 mg/dL- corneal opacification, kidney disease

Treatment of Severe HDL Deficiency

- Make the diagnosis
- Optimize all risk factors
- Treat the underlying condition

Genes for Lipid Disorders

- LDLR, APOB, LDLRAP1, PCSK9, ABCG5/8, CYP27A1 (High LDL-C and High Sterols)
- APOA5, APOC2, APOC3, GPIHBP1, LPL, LMF1 (High TG)
- ABCA1, APOA1, LCAT (Low HDL)
- CETP, LIPC, SAR1B, SCARB1 (High Dysfunctional HDL)
- APOE, ANGPTL3, GPD1, LIPA (Combined Hyperlipidemia, Dysbetalipoproteinemia, Cholesteryl Ester Storage Disease or Lysosomal Acid Lipase Deficiency)
- APOB, MTTP (Very Low LDL)

Emory Genetics Laboratory
Thank you for your attention