Genetics of Hypertriglyceridemia and Emerging Therapies

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Triglyceride and Genetics in History

1850
Michel-Eugène Chevreul 1786-1889

Johann Gregor Mendel 1822-1884

1953
Donald Sharp Fredrickson

James D. Watson and Francis Crick
The Majority of the Classical Fredrickson phenotypes are Associated with HyperTG and have a shared genetic architecture

<table>
<thead>
<tr>
<th>TYPE</th>
<th>LIPOPROTEIN</th>
<th>HyperTG</th>
<th>FASTING TG VALUES</th>
<th>GENETIC BASIS</th>
<th>KEY GENES</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>CM</td>
<td>Very severe hyperTG</td>
<td>&gt;1000 mg/dl</td>
<td>Rare autosomal recessive disease</td>
<td>Rare variants in LPL, apoC-2, apoA-5, GPIHPB1, LMF-1 genes</td>
</tr>
<tr>
<td>IIA</td>
<td>LDL</td>
<td>No</td>
<td>&lt; 150 mg/dl</td>
<td>Common mendelian forms (eg: FH)</td>
<td>LDLR, apoB, PCSK9, ARH, apoE</td>
</tr>
<tr>
<td>IIB</td>
<td>LDL, VLDL</td>
<td>Moderate hyperTG</td>
<td>200-450 mg/dl</td>
<td>&gt;15% of FH patients</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>IDL</td>
<td>Moderate or severe hyperTG</td>
<td>&gt;200 mg/dl</td>
<td>Strong mendelian susceptibility</td>
<td>apoE2, LIPC</td>
</tr>
<tr>
<td>IV</td>
<td>VLDL</td>
<td>Moderate or severe hyperTG</td>
<td>HIGH &gt; 200 mg/dl</td>
<td>Complex and common trait</td>
<td>38 Genes (at least)</td>
</tr>
<tr>
<td>V</td>
<td>CM, VLDL</td>
<td>Very severe hyperTG</td>
<td>&gt;1000 mg/dl</td>
<td>Oligogenic</td>
<td>LPL, apoA-5, others</td>
</tr>
</tbody>
</table>
# Genetic Architecture of TG-related Phenotypes Beyond the Frederickson’s Classification

<table>
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<tr>
<th>TYPE</th>
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<th>FASTING TG</th>
<th>GENETIC BASIS</th>
<th>KEY GENES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp(a)</td>
<td>Pre-beta VLDL</td>
<td>Variable</td>
<td>Mendelian or oligogenic</td>
<td>Apo(a)</td>
</tr>
<tr>
<td>hypoalphalipoproteinemia</td>
<td>HDL</td>
<td>depends on intercellular signals and plasma TG exchange</td>
<td>Mendelian forms exist</td>
<td>CETP, LCAT, and others</td>
</tr>
<tr>
<td>Impaired fat tolerance</td>
<td>CM remnants</td>
<td>Normal Fasting, High post prandial TG peak and AUC</td>
<td>oligogenic</td>
<td>Common LPL loss-of-fonction variants</td>
</tr>
<tr>
<td>a(hypo)beta lipoproteinemia</td>
<td>all</td>
<td>Normal or hypoTG (&lt;100 mg/dl)</td>
<td>mendelian</td>
<td>MTP and others</td>
</tr>
<tr>
<td>Hyperglycerolemia and dysglycerolemia</td>
<td>None (free glycerol)</td>
<td>350-1000 mg/dl</td>
<td>X-linked</td>
<td>GK 19 others</td>
</tr>
<tr>
<td>MCT</td>
<td>None (CM independant)</td>
<td>variable</td>
<td>Diet-dependent</td>
<td></td>
</tr>
<tr>
<td>Lipodystrophies (Type 1 to 4)</td>
<td>N/A</td>
<td>200-1000 mg/dl</td>
<td>Mendelian and acquired forms (eg: AIDS)</td>
<td>8 genes identified: AGPAT2, BSCL2, CAV1, PTRF, PPARG, v-AKT, Lamin A/C,LMF1</td>
</tr>
</tbody>
</table>
Genetic Testing

Why studying the genetic architecture of hyperTG and why developing genetic tests?

- To diagnose rare mendelian causes of hyperTG;
- To confirm the diagnosis;
- For the screening of carriers of mendelian diseases;
- To identify strong susceptibility to common diseases (high RR);
- To improve our knowledge of rare and common diseases;
- For risk stratification and risk assessment (RR, PAR and mendelian randomization);
- To identify novel targets for treatments;
- To understand drug or diet response and tolerability;
For each intended use

Four components of evaluation (ACCE)
- Analytic Validity
- Clinical Validity
- Clinical Utility
- ELSI

Analytic framework – 40+ targeted questions

http://www.cdc.gov/genomics/activities/fbr.htm
The Genetic Architecture of Hypertriglycerideridemia is more complex than that of Hypercholesterolemia

- The risk of disease is not linear across plasma TG concentration distribution;

- TG values are highly variable (post-prandially, daily, seasonally, across the life span);

- Several different diseases (rare or common) are associated with hyperTG (hyperTG being either causal or a consequence);

- The association of HyperTG with the risk of disease is not limited to CVD.
The Genetic Architecture of Hypertriglyceridemia is more complex than that of Hypercholesterolemia

The TG « number » behind the scene:

- The enzymatic method used for plasma TG determination measures glycerol (the sum of free glycerol, MAG, DAG and TG) not TG only;

- The nature of the fatty acids (FA) bound to glycerol in the acylglycerol molecule influences both the acylglycerol structure and health status (trans-FA, MUFA, PUFA, omega-3, omega-6, etc...)
The Genetic Architecture of Hypertriglyceridemia is more complex than that of Hypercholesterolemia

- There are two main TG biosynthetic pathways, the sn-glycerol-3-phosphate pathway, which predominates in liver and adipose tissue, and the monoacylglycerol pathway in the enterocyte.

- There are two different paths for TG transportation and delivery: the exogenous (Chylomicron) and the endogenous (VLDL) pathways;
(A) Exogenous (chylomicron) Pathway

- Chylomicron (CM)
- LPL

(1)

(2)

adipocytes

Muscle cells

(B) Endogenous (VLDL) Pathway

- Liver
- Peripheral cells
- VLDL
- LDL
- IDL
- FFA
Systems and Molecular Approaches required to study the genetic basis of Hypertriglycerideremia

**DOMAINS**
- Epigenome
- Genome
- Transcriptome
- Proteome/metabolome/interactome
- Lipoproteins phenome/human phenomes
- Sociome

**TARGET**
- Genes/mutations/epimutations
- mRNA transcripts
- Proteins/networks
- Phenotypes/individuals
- Population/community systems

**APPLICATIONS**
- GWAS/NGS
- Fine Mapping
- Sequencing (common and extremes)
- Functional Omics (in vitro, in vivo)
- Clinical Sciences
- Public Health
# TG-Associated Genes Identified by Various Methodologies

<table>
<thead>
<tr>
<th>Locus</th>
<th>Identified by GWAS of HTG</th>
<th>Replication of population based TG-associated loci</th>
<th>HTG-causing rare variants</th>
<th>Mouse models of HTG</th>
<th>Family based and linkage studies</th>
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</thead>
<tbody>
<tr>
<td>APOA5</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>GCKR</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>LPL</td>
<td>X</td>
<td>X</td>
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<tr>
<td>APOB</td>
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<tr>
<td>APOE</td>
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<td>X</td>
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<td>ANGPTL3</td>
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<td>MLXIPL</td>
<td>X</td>
<td></td>
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<td>TRIB1</td>
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<td>NCAN</td>
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<td>GALNT2</td>
<td></td>
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<td></td>
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<td>LIPC</td>
<td></td>
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<tr>
<td>APOC2</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
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<tr>
<td>GPIHBP1</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
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<td>LMF1</td>
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<td>X</td>
<td>X</td>
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</tr>
<tr>
<td>USF1</td>
<td></td>
<td></td>
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<td></td>
<td>X</td>
</tr>
</tbody>
</table>

Adapted from Johansen et al. J Lipid Res 2011;52:189-206
Proportion of Variation Explained by Clinical Variables, Common Variants and Rare Variants in HyperTG-Associated Genes

Clinical variables: age, sex, BMI and diabetes
Common variants: APOA5, GCKR, LPL, APOB, MLXIPL, TRIB1, ANGPTL3, NCAN/CILP2, FADS1-2-3, XKIR6/PINX1, PLTP

Resequencing of extremes involves deep sequencing of two groups of individuals, one at each extreme of the phenotype.

It is usually used to evaluate genomic regions with a high suspicion of association or to analyse potential rare causes of severe hyperTG.

The associations detected are more likely to reflect causative variants of larger effect size (RR) than GWAS.

Rare causative mutations, identified by resequencing might facilitate the identification of frequent variants.
Gene x Gene and Gene x Environment Interaction Between Frequent HyperTG-Related Gene Variants Explain a Larger Proportion of TG Variance

Plasma Triglyceride Concentration in Relation to Obesity and Four Common Gene Variants in the VLDL Pathway (APOB, LIPC, PPARA, LPL)

- Men: < 90 cm
  - Women: < 85 cm
- Men: ≥ 90 cm
  - Women: ≥ 85 cm

Plasma triglyceride (mmol/L)

Combination

- Combination 0
- Combination 2
- Combination 1
- Combination 3+

Waist circumference

Men: < 90 cm
Women: < 85 cm

Men: ≥ 90 cm
Women: ≥ 85 cm

p = 0.011
Severe HyperTG: Frequency of Carriers of APOA5 Variants According to Quartile of Plasma Triglycerides

Mendelian Randomization to Assess the Association of HyperTG with Disease

- Mendelian randomization is a method using unbiased estimates of variation in genes of known function to study the causal effect of a modifiable exposure on disease, without conducting a traditional randomized trial.

- Misleading conclusions can be drawn in the presence of:
  - linkage desequilibrium
  - Genetic heterogeneity
  - Pleiotropy
  - Population stratification
APOA5 and Mendelian Randomization to Assess the Association of HyperTG with CHD

- The Triglyceride Coronary Disease Genetics Consortium and Emerging Risk Factors Collaboration has used mendelian randomization to study Triglyceride-mediated pathways and CHD (*Lancet* 2010; 375:1634-1639).

- The theory behind this study was that APOA5 genetic variant specifically affects triglyceride concentrations and only minimally affects levels of other lipids, thus mimic a randomized trial.
APOA5 and Mendelian Randomization to Assess the Association of HyperTG with CHD

- In this study, each copy of the minor APOA5 allele raised mean triglyceride concentration by 16%. There was no significant effect on LDL-C and minor effect on HDL-C.

- Analysis of case–control studies showed a highly significant 18% increase in CHD (odds ratio of 1.18) for every minor APOA5 allele carried;

- This suggests an effect of triglycerides on CHD that is not mediated by LDL-C or HDL-C.

- Limitations
Risk Associated with HyperTG: the « ROC POP » Approach

- The “ROC POP” approaches uses a sequence of experimental approaches to establish the risk and achieve clinical benefit:
  - from Rare and Orphan extreme forms of hyperTG -> to Common, less severe hyperTG phenotypes –> to Population health
  - Resequencing of extremes
  - study of rare causes of very severe HyperTG
Causes of Very Severe (>900 mg/dl) HyperTG

- **Familial Chylomicronemia Syndrome (FCS) or Type I**
  - Rare: less than $2 \times 10^6$
  - Associated with complete LPL deficiency
  - Autosomal recessive disease (loss-of-function mutations in the LPL, APOA5, APOC2, GPIHBP1 or LMF1 genes)

- **Non-FCS**
  - Estimated Prevalence: 1/600
  - Type V (oligogenic)
  - severe Type III (apoE2)
  - Severe type IV (complex trait)
  - Others (eg:GKD)
Risk of Pancreatitis Associated with HyperTG

- Moderate Hyper TG (5.0-9.0 mmol/l) n = 487
- Of any cause n = 354
- Severe Hyper TG (> 9.0 mmol/l) n = 354

Patients with elevated fasting TGs

Pancreatitis risk relative to normal population

Odds ratio ± SE (p-value)

361.4 ± 401.2
(< 0.001)

55.8 ± 56.9
(< 0.001)

15.8 ± 16.3
(0.008)

LPLD patients n = 28

Patients with elevated fasting TGs
FCS Clinical Expression

- Presentation with abdominal pain, eruptive xanthoma, hepatosplenomegaly, lipemia retinalis, milky (lactescent) appearance of the plasma;

- Complication of recurrent acute pancreatitis and pancreatic insufficiency.

- Not responding to usual LLD
## Risk Associated with Very Severe (>900 mg/dl) HyperTG

<table>
<thead>
<tr>
<th>Fredrickson’s nearest phenotype (etiology)</th>
<th>Type I (familial chylomicronemia)</th>
<th>Type III (apo E resistance)</th>
<th>Type IV (secondary causes dominate)</th>
<th>Type V (oligogenic)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FCS</strong></td>
<td>OR [95% CI]</td>
<td>OR [95% CI]</td>
<td>OR [95% CI]</td>
<td>OR [95% CI]</td>
</tr>
<tr>
<td><strong>Non FCS</strong></td>
<td>p-value</td>
<td>p-value</td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>Co-morbidities a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obesity (BMI ≥ 30 kg/m²)</td>
<td>0.58 [0.13-2.58]</td>
<td>4.01 [2.12-7.57]</td>
<td>3.41 [2.13-5.47]</td>
<td>4.68 [2.71-8.09]</td>
</tr>
<tr>
<td></td>
<td>0.472</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Coronary Artery Disease</td>
<td>0.65 [0.14-3.12]</td>
<td>1.46 [0.67-3.15]</td>
<td>1.70 [0.99-2.92]</td>
<td>1.94 [1.03-3.66]</td>
</tr>
<tr>
<td></td>
<td>0.589</td>
<td>0.338</td>
<td>0.056</td>
<td>0.041</td>
</tr>
<tr>
<td>Peripheral Artery Disease</td>
<td>NA NA</td>
<td>19.42 [2.06-182.86]</td>
<td>1.56 [0.10-25.46]</td>
<td>NA NA</td>
</tr>
<tr>
<td></td>
<td>0.010</td>
<td>0.753</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial Hypertension</td>
<td>1.10 [0.39-3.06]</td>
<td>0.72 [0.36-1.43]</td>
<td>1.21 [0.77-1.92]</td>
<td>1.96 [1.13-3.40]</td>
</tr>
<tr>
<td></td>
<td>0.863</td>
<td>0.351</td>
<td>0.408</td>
<td>0.017</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3.19 [0.93-10.97]</td>
<td>1.84 [0.80-4.25]</td>
<td>3.58 [2.05-6.25]</td>
<td>6.50 [3.47-12.15]</td>
</tr>
<tr>
<td></td>
<td>0.066</td>
<td>0.154</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>0.017</td>
<td>0.042</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>361.40 [41.06-3181.20]</td>
<td>22.63 [2.23-222.18]</td>
<td>38.28 [4.87-300.70]</td>
<td>54.43 [6.82-434.47]</td>
</tr>
<tr>
<td></td>
<td>&lt; 0.001</td>
<td>0.008</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Notes:

- **Co-morbidities a**
  - OR: Odds Ratio
  - 95% CI: 95% Confidence Interval
  - p-value: Statistical significance

- **Columns**
  - **FCS**
  - **Non FCS**

- **Risk Factors**
  - Obesity
  - Coronary Artery Disease
  - Peripheral Artery Disease
  - Arterial Hypertension
  - Diabetes
  - Glucose Intolerance
  - Pancreatitis

- **Statin Use**
  - Risk associated with very severe (>900 mg/dl) hypertriglyceridemia (HyperTG) in different phenotypes of Fredrickson's lipoprotein profile.
## Risk Associated with Very Severe (>900 mg/dl) HyperTG

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The challenge is to identify a genetic and phenotypic discrimination limit which will separate the distributions of patients with severe (>900 mg/dl) hyperTG due to FCS vs those without FCS.

**Rule-out FCS** (rule-in CAD and cardiometabolic risk)

**Rule-in FCS** (pancreatitis risk)

The « ROC POP » Approach of Very Severe HyperTG

Plasma TG Distribution in the population

- Reference limits are derived cutpoints based on the distribution of TG values in a Reference Sample

- TG Value (mg/dl)
  - 150
  - 95th percentile
  - 99th percentile
  - 99.8th percentile
  - 900
  - 99.8th percentile

Patients with severe (>900 mg/dl) hyperTG

- Patients without FCS
- Patients with FCS

Biomarkers Value

The « ROC POP » Approach of Very Severe HyperTG

- The challenge is to identify a genetic and phenotypic discrimination limit which will separate the distributions of patients with severe (>900 mg/dl) hyperTG due to FCS vs those without FCS.
Emerging Therapies for HyperTG

- The « ROC POP » sequential strategy for drug development:
  - Evaluate the efficacy and safety of emerging therapies in rare, orphan, extreme forms of very severe hyperTG
  - If the treatment is not developed for orphan designation only, then assess the treatment in less severe forms;
  - Then assess the treatment in common forms.
Emerging Treatments of Severe HyperTG Across the “Omics” Spectrum

- **EMERGING TREATMENTS**
  - Gene Replacement Therapy
  - RNA-Based Therapy
  - Cell and Pathway Pharmacology
  - Clinical Sciences Genetic and Pharmacogenetic Testing
  - Health Promotion Disease Prevention Genetic Screening

- **DOMAINS**
  - Epigenome
  - Genome
  - Transcriptome
  - Proteome/metabolome/interactome
  - Lipoproteins phenome/human phenomes
  - Sociome

- **TARGETS**
  - Genes/mutations/epimutations
  - mRNA transcripts
  - Proteins/networks
  - Phenotypes/individuals
  - Population/community systems

- **FUNCTIONNAL FOODS**
  - Behavioral Approaches
  - Life Habits

- **EPI-MEDICATION**
Potential targets for gene replacement therapy: LPL, apoC2, ApoA-5, GPIHBP1

Potential targets for anti-sense therapy: apoB, apoC-III, DGAT1, PCSK9

MicroRNAs
Aptamers

Cell Pathways:
Peptide linker technologies

metabolic pathways: DGAT-1inh, MTPI, PCSK9 inh

Peptide-based mimetics:
HDL, apoE, etc
Glybera: AAV2-LPL$^{S447X}$ Gene Replacement Therapy
Glybera Gene Replacement Therapy for LPL Deficiency

- First Gene replacement therapy being authorized in the occidental world
- Designed for patients with FCS due to loss-of-function LPL gene mutations.
- Not for FCS caused by apoA-5, apoC-2, GPIHPB1, LMF-1 gene mutations.
- Requires genotyping (genetic test)
Glybera Mechanism of Action
Fasting TG Decreased After 12 Weeks of Treatment but Returned to Baseline After 5 Months
Ultracentrifugation Data at > 5 Months Suggest Sustained Changes in TG-rich Lipoproteins Composition

- Diminution of the number and size of particles in the chylomicrons stratum
- Shift of TG from the CM fraction to the sf 20-400 fraction
Results shown are a mean ± SEM; n=5 (wk-2 and wk+14) or n=3 (wk+52)

p-values: t-test AUC_{24hrs}; wk -2 versus wk +14 and wk +52
**ISIS-APOCIII<sub>Rx</sub> Anti-Sense Therapy**

- Structure: 20-nucleotide (20-mer) antisense oligonucleotide (ASO)
- Complementary, specific ASO sequence that crosses the hepatocyte cell membrane and binds in coding region of mRNA for ApoC-III

![Diagram showing the mechanism of action of ISIS-APOCIII<sub>Rx</sub> anti-sense therapy](adapted_from_Crooke_R. Antisense_oligonucleotides_as_therapeutics_for_hyperlipidaemias. _Expert_Opin._Biol._Ther._(2005), 5(7).)

Apolipoprotein C-III is a Key regulator of Lipid Metabolism

- ApoC-III is a 79 amino acid glycoprotein
- Links to apoB-containing lipoproteins and HDL
- Potent inhibitor of LPL-catalyzed lipolysis of TG-rich lipoproteins
- Inhibits hepatic lipase which also plays an important role in the conversion of VLDL to IDL
- Inhibits receptor- and non-receptor-mediated uptake of lipoprotein remnants by the liver
- Independent risk factor for cardiovascular disease
ISIS-APOCIII$_{Rx}$ Anti-Sense Therapy in Patients with FCS

- ISIS-APOCIII$_{Rx}$ is mainly active in the liver: will it affect chylomicron metabolism?

- Apo CIII is a potent inhibitor of LPL-catalyzed lipolysis of TG-rich lipoproteins. Can we expect that ISIS-APOCIII$_{Rx}$ will improve the status of patients having undetectable LPL activity?

- A PoC study has been executed to answer these questions.
Phase 2 Multicenter Randomized Double-Blind Placebo Controlled Study in High to Severely High TG Patients

**COHORTS**

**MONOTHERAPY**
- Patients not on TG-lowering therapy with TG levels ≥440 & ≤2000 mg/dL
  - 100, 200, 300 mg
  - 3:1 (active: placebo)
- Postprandial assessments

**ADD-ON TO FIBRATE**
- Patients on stable dose fibrate TG levels ≥225 & ≤2000 mg/dL
  - 200, 300 mg
  - 2:1 (active: placebo)

**FCS**
- FCS patients with TG levels ≥1500 mg/dL
  - 300 mg
  - Open label
- Postprandial CM characteristics, kinetics and metabolism

**Treatment Period**
- 13 Weeks

**Post-Treatment f/u Period**
- 13 weeks

**Screen/**
- D1 D8 D15 D22 D29 D36 D43 D50 D57 D64 D71 D78 D85

**≤8 weeks**
ISIS-APOCIII_Rx Treatment Reduced Fasting ApoC-III Levels in FCS Patients (Absolute Levels)
ISIS-APOCIII\textsubscript{Rx} Treatment Reduced Fasting Triglyceride Levels in FCS Patients (Absolute Levels)

Fasting Triglyceride Levels

- Patient #1
- Patient #2
- Patient #3
ISIS-APOCIII<sub>Rx</sub> Treatment Reduced Fasting Non-HDL-C Levels in FCS Patients (Absolute Levels)
ISIS-APOCIII$_{Rx}$ Treatment Improved Overall Lipid Profile in FCS Patients (Mean % Change)
Emerging Therapies for Very Severe HyperTG and FCS: Metabolic Pathways and Cell Pathway Pharmacology

- Novartis LCQ908 (DGAT-inhibitor)
- Catabasis CT-2003 (linker technology)
- Functional FCS-adapted foods
- Aegerion Lomitapide (MTPI)
  http://www.aegerion.com
- Others
DGAT1, Chylomicron production and Pathophysiology of the Familial Chylomicronemia Syndrome (FCS)

Small Intestine

- Triglyceride (TAG)
  - MAG
  - DAG
  - TAG
  - DGAT1
  - MAG
  - FFA
  - FFA

Absorptive enterocyte

- PANcreatic lipase

Vascular endothelium

- FFA FFA FFA
- MAG DAG TAG
- FACoA FACoA
- FFA FFA FFA

Chylomicron

- LPL

Bloodstream

- Chylomicron

Lymph

Vascular endothelium

Skeletal muscle & Adipose Tissue

Fasting TG (mg/dL):
- Healthy controls: 88
- Moderate hyperTG: 566
- Other Chylomicronemia: 1434
- FCS: 2739

Odds Ratio

FCS: 361.4
DGAT Biochemical Pathway

Enzymes

AGPAT2 = 1-acetylgllycerol-3-phosphate O-acyltransferase 2 (lysophosphatidic acid methyltransferase)
ARAT = Acyl-CoA retinol acyltransferase
ATGL = Adipose-tissue TG lipase
CDIPT = CDP-diacylglycerol–inositol 3-phosphatidylinositol transferase
GPAM = Glycerol 3-phosphate acyltransferase, mitochondrial
HSL = Hormone-sensitive lipase
MGL = Monoacylglycerol lipase
PAP = Phosphatidic acid phosphatase

Dietary triglycerides

- Pancreatic Lipase
  - Fatty acid (FA)
  - Glycerol
  - Mono/diacylglycerol

Dietary glucose

- FA synthesis

Glycerol

Pathway present in most cells

- Glycerol Kinase
  - Glycerol-3-phosphate

- FA CoA
  - GPAM

Phosphatidate

- AGPAT2
  - Phosphatidate

- PAP

Monoacylglycerol

- FA
  - HSL

Diacylglycerol

- CDIPT
  - Phospholipid synthesis

- DGAT1
  - MGAT activity

Triacylglycerol

- ATGL or HSL

Remnants

- Lipoprotein Lipase
  - Lipoprotein
  - Lipid droplet

Alternate pathways

- DGAT1
  - Wax synthase activity

Fatty acyl alcohols

- DGAT1
  - Mono/diester waxes

Retinol

- DGAT1
  - Retinyl esters
  - ARAT activity

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Results of this PoC study have been presented at the NLA 2012 meeting.

A multicenter, international, double-blind, placebo controlled phase 3 study involving 50 FCS patients is ongoing.

http://clinicaltrials.gov/show/NCT01514461
Fasting Triglyceride Levels During Treatment with LCQ908

Period 1: 20 mg
- Very low fat diet (<15%)
- Daily LCQ908 treatment
- Day -7, 1, 7, 14, 21

Period 2: 40 mg
- Very low fat diet (<15%)
- Daily LCQ908 treatment
- Day -7, 1, 7, 14, 21

Period 3: 10 mg
- Very low fat diet (<15%)
- Daily LCQ908 treatment
- Day -7, 1, 7, 14, 21

Geometric mean ± SEM
Effects of LCQ908 (20 mg) on Triglyceride Content in Lipoprotein Fractions

mean ± SEM
SMART Linker Conjugates
- Proprietary linker technology used to construct bifunctional compounds that target a disease pathway at multiple points

Efficacy
- Focus on key disease targets
- Match PK of bioactives
- Produce mechanistic synergy
- Increase intracellular omega 3 concentration

Tolerability and Safety
- Pathway targeted compound is inactive until released inside the targeted cell

TG Pathways

SMART Linker Conjugate

Enzyme Specific Cleavage of SMART Linker
Release of Bioactives

Multi-pronged pathway modulation

Cell

A = Bioactive A targets a node in a disease pathway
B = Bioactive B targets a node in a disease pathway
CAT-2003: Inactive Until Entering the Target Cell

- Intracellular CAT-2003 synergistic efficacy in intestine and liver

In CAT-2003, niacin and EPA are linked in a way that delivers both intracellularly preventing interaction with extracellular receptors.

Flushing

Extracellular GPR-109A Receptor Mediates Flush and Free Fatty Acid Suppression
CAT-2003 Activates Lipoprotein Lipase (LPL) to Accelerate Triglyceride Clearance

CAT-2003 inhibits the production of the negative regulators of LPL through activated SREBP and reductions in PCSK9

Angptl3/4: angiopoeitin-like protein 3/4
CAT-2003 Clinical Status

**Phase 1:**
- Established safety and tolerability of single and multiple doses
  - No safety issues
  - No flushing
  - Tolerability acceptable in range where pharmacology demonstrated
- Established clinical exposure
- Proof of concept pharmacological effects
  - Reductions in apo B-containing lipoproteins and PCSK9
  - Profound reductions in post-prandial triglycerides
  - Reductions in fasting triglycerides in hypertriglyceridemic subjects

**Phase 2: in progress**

http://www.catabasis.com/
Severe HyperTG: Lessons Learned From Genetics and Emerging Therapies

- The mechanisms and signals involved in TG-rich lipoproteins metabolism and cell uptake are complex, sophisticated and still incompletely understood;

- TG-rich lipoprotein metabolism and kinetics can be improved without decreasing plasma TG values, including in patients with LPL deficiency and TG values > 1000 mg/dl.

- Preliminary results suggest that it is possible to significantly decrease plasma TG and CM-TG concentration in absence of functional LPL;

- It is possible to up-regulate lipolysis without increasing LPL mRNA

- The contribution of apoC-3 in TG-rich lipoprotein metabolism, trafficking and signalling represent a fascinating challenge;
Severe HyperTG: Future directions

- Functional analyses of emerging genetic markers identified by GWAS or other strategies identify new transcriptional and post-transcriptional regulation of TG-rich lipoprotein metabolism. Among which:

  - Angiopoietin-like proteins (ANGPTL3 and 4), sterol regulatory element binding protein 1 (SREBP-1), LXR and mTORC complex role in the transcriptional regulation of lipid biosynthesis and ApoA-5 dependent LPL activation or lipolysis;
  - Post-transcriptional regulation of lipid and TG-rich lipoproteins metabolism by extracellular and cellular non coding microRNAs
  - Role of Sorting receptors (sortilins and SORTA)
  - Role of PCSK9 and endocytic receptors such as the syndecan-1 heparan sulfate proteoglycan (HSPG) or docking receptors such as SR B1

- The genetic dissection of hyperTG and its risk trajectory remains a real Christmas tree... and a challenge.