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- Drs. Dent, Hsu, Wasserman, and Mr. Davis: employees, stockholders of Amgen Inc.
A Genome-Wide Association Study (GWAS) Identifies Novel Loci Associated with Clinically Defined Statin-Associated Muscle Symptoms in the Double-Blind Placebo-Controlled GAUSS-3 Trial

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Statin-Associated Muscle Symptoms (SAMS)

- Patients with high Cardiovascular risk are more likely to discontinue statin use after experiencing SAMS.

- Diagnosis of SAMS is based on subjective complaints; most patients do not exhibit CK- or other lab abnormalities.

- Pathophysiological mechanisms contributing to SAMS are largely unknown.

- The true incidence of SAMS has been questioned due to conflicting rates of SAMS in observational studies versus randomized trials.
Genetic Variations and Muscle Toxicity

- Plasma statin concentrations are influenced by genetic variations which may also influence the likelihood of statin side effects

- **SLCO1B1** mediates hepatic uptake of statins
  
  - Coding variant in **SLCO1B1** (rs4149056) associates with increased risk of myonecrosis

- No genetic association performed for SAMS

*SLCO1B1* = solute carrier organic anion transporter family, member 1B1

**Serum [statin] associated with coding variant rs4149056 (Val174Ala) in SLCO1B1 gene**

Objective

• To identify genetic variants that contribute to the risk of SAMS via an exploratory genome-wide screening in participants from the GAUSS-3 trial
The GAUSS-3 Study Design

Phase A

511 patients enrolled at 53 centers with a history of muscle symptoms to multiple statins.

10 weeks

Atorvastatin 20 mg
Placebo

10 weeks

Atorvastatin 20 mg
Placebo

Phase B

Patients proceeded to Phase B only if they had *intolerable* muscle symptoms on atorvastatin, but not placebo, or CK ≥ 10 x ULN during prior statin treatment

24 weeks

Monthly SC evolocumab 420 mg
Daily oral ezetimibe 10 mg

Selection of Samples

- SAMS was confirmed in 159 patients in GAUSS-3
- 747 controls on stable statin dose in other evolocumab trials or failed screening on re-challenge for SAMS in the GAUSS-3 study

<table>
<thead>
<tr>
<th>STUDY</th>
<th>PHENOTYPE</th>
<th>SUBJECTS (N)</th>
<th>SAMPLES (n)</th>
<th>GENOTYPED* (n)</th>
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</thead>
<tbody>
<tr>
<td>GAUSS-3</td>
<td>Statin Rechallenge for Intolerance SAMS Confirmed</td>
<td>500</td>
<td>434</td>
<td>159</td>
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<tr>
<td></td>
<td>Statin Rechallenge for Intolerance Screen Fails</td>
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<tr>
<td>RUTHERFORD</td>
<td>HeFH on a Stable Dose of Statin + other lipid-lowering therapies</td>
<td>168</td>
<td>146</td>
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<tr>
<td>RUTHERFORD-2</td>
<td>HeFH on a Stable Dose of Statin + other lipid-lowering therapies</td>
<td>329</td>
<td>53</td>
<td>31</td>
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<tr>
<td>LAPLACE-TIMI 57</td>
<td>Stable Dose of Statins</td>
<td>631</td>
<td>360</td>
<td>356</td>
</tr>
</tbody>
</table>

*All samples meeting consent review and QC parameters were analyzed
†Adult Caucasians selected from 159 total cases and 747 total controls
Methods

- **Genotyping:** Illumina OmniExpressExome-8 v1.3
  730K common variants: 228K missense/nonsense variants

- **Imputation:** Haplotype Reference Consortium database (*35,000 whole genome sequences*) used to impute un-genotyped SNPs up to 40 million SNPs:
  - 25,000 protein coding genes, non-coding RNA and non-coding regions

- **Statistical modeling:**
  - Genetic model: additive and recessive models
  - Single variant associations: minor allele count (MAC) > 5

- **Covariates:** Age, sex, BMI, ancestral genetic background
Manhattan Plot Identifies 3 Genome-Wide Significant Loci of SAMS

5x10^{-8}

5x10^{-6}

GW Significant

GW Suggestive

MGAT5

KCNJ2 / SOX9

MRPS6 / ATP5O

p-value
**The MRPS6 / ATP5O Locus**

- SNP chr21:35375595 (MAF = 35%) associated with decreased risk of SAMS
  - OR = 0.5 (95%CI: 0.4 ~0.7) per risk allele
MRPS6 / ATP50

• **MRPS6** and **ATP50** involved in mitochondrial function
  
  – **MRPS6 gene**: Mitochondrial Ribosomal Protein S6
    • **Experimental**: MRPS family regulates translation of mitochondrial proteins: MRPS inhibition reduces mitochondrial energy production¹
    • **Clinical**: Variants associated with CHD and early onset MI
  
  – **ATP50 gene**: Mitochondrial ATP Synthase Complex V
    • **Experimental**: ATP production via mitochondrial respiratory chain

• Statins suggested to impact muscle function by interference with mitochondrial function²

The *MGAT5* Locus

- SNP rs6714520 (MAF = 17%) associated with an increased risk of SAMS
  - OR = 2.2 (95%CI: 1.7 ~3.0) per risk allele

- *MGAT5* codes for Mannosyl (Alpha-1,6-)Glycoprotein Beta-1, 6-N-Acetyl-Glucosaminyltransferase
  - Key enzyme involved in the regulation of the biosynthesis of glycoprotein oligosaccharides

- *Mgat5*-deficient mice:
  - Display reduced glucose metabolism in skeletal muscle, fewer muscle satellite cells, and accelerated loss of muscle

No Relation Between Candidate Genes Involved in Statin Metabolism and SAMS

- 20 candidate genes hypothesized to play a role in drug metabolism, myopathy, and myalgia
- Candidate genes were not associated with SAMS, after correction for multiple testing ($P_{\text{corrected}} < 1.6 \times 10^{-4}$)

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Alleles</th>
<th>Effect Allele</th>
<th>Function Role</th>
<th>Protein Coding</th>
<th>Nominal P*</th>
<th>ORs</th>
<th>95% CI</th>
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<td>UGT1A3</td>
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<td>A</td>
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<td>1.59E-03</td>
<td>2.1</td>
<td>1.3 – 3.4</td>
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<td>C</td>
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<td>1.2 – 3.1</td>
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<td>0.5 – 0.9</td>
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<td>G</td>
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<td></td>
<td>0.02</td>
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<td>1.1 – 2.6</td>
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<tr>
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<td>rs4149056†</td>
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<td>0.6</td>
<td>0.4 – 1.0</td>
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</tbody>
</table>

*Nominal P < 0.05 displayed
†rs4149056 reported to associate with serum statin levels
Limitations

- The ‘controls’ may have been diluted, as ‘screen-failed’ intolerant subjects were included as controls.  
  - Introduces misclassification towards null hypotheses

- The GAUSS-3 Trial is the largest study using a placebo-controlled, statin re-challenge  
  - Validation of our findings require an independent patient population with ‘blindend rechallenge confirmed’ SAMS, which is unavailable

- This analysis was confined to Caucasian subjects; limiting translation to an ethnically diverse population.
Summary

• This hypothesis-generating GWAS study identified novel loci in patients with objectively-identified clinical phenotype for SAMS:
  - MRPS6/ATP5O locus – Decreased risk of SAMS
  - MGAT5 gene and KCNJ2/SOX9 loci – Increased risk of SAMS

• As candidate genes are related to mitochondrial respiration, mitochondrial function may link statins and SAMS.

• Genes reported to influence plasma statin concentration were not significantly associated with SAMS.
Closing Thoughts

• Although exploratory, GWAS findings may provide new opportunities to explore the biological basis of SAMS.

• Pending validation, GWAS findings may help design polygenic risk score model to identify patients more likely to have ‘true’ SAMS.
Stay Tuned!

Thank you for your attention