Genetic risk determination of hypercholesterolemia and coronary artery disease at the individual patient level

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Context: Atherosclerotic cardiovascular disease (ASCVD) is the leading cause of mortality, globally. ASCVD is a heterogeneous disease with multiple, interacting, pathways contributing to its etiology. Hypercholesterolemia is one major independent risk factor for ASCVD. Other pathways that work additively to hypercholesterolemia to increase ASCVD risk include: high lipoprotein(a), hypertriglyceridemia, combined hyperlipidemias, defects in lipoprotein remnant clearance, hypoalphalipoproteinemia, reverse cholesterol transport (RCT) defects and inflammation and others.

Methods: We have developed GBinsight, a next-generation sequencing (NGS)-based genetic test that analyzes coding and regulatory regions of genes reported to affect these pathways to comprehensively assess ASCVD risk. With expanded genomic coverage, GBinsight can compute both monogenic and polygenic causes, which is elemental for risk stratification. Results: Herein, we report on our findings from 55 patients from three academic-based preventive cardiology/lipid clinics that present with severe hypercholesterolemia (≥190mg/dl). We identified 22 (40%) with a known monogenic cause and another 21 (38%) with a polygenic cause. Additionally, we identified 31% of our cohort with a variant that increases LP(a) levels. Another 7 patients had a rare variant in a gene that affects the RCT pathway. Conclusions: Genetic causes of hypercholesterolemia are heterogeneous. Furthermore, a more comprehensive analysis of other pathways that may increase ASCVD may have clinical utility. GBinsight analyzes both monogenic and polygenic causes, which is elemental for risk stratification and precision medicine applications.

Introduction

Multiple pathways interact to affect ASCVD risk. Genetic studies that assess a polygenic risk suggest that risk in multiple pathways are additive in predicting ultimate risk of ASCVD$^1$. We have developed GBinsight Comprehensive panels with the understanding of this complexity in mind. Figure 1 schematically shows the interplay of genetic risk across multiple pathways and affecting ASCVD risk and its potential clinical application in risk stratification and patient management (figure 2).

Figure 1. Heterogeneity of pathways contributing to atherosclerotic cardiovascular disease (ASCVD) and a selection of genes covered by the GBinsight Comprehensive Dyslipidemia panel.
Comprehensive genetic assessment of hypercholesterolemia and coronary artery disease risk

Table 1. Genes analyzed by GBinsight Comprehensive Dyslipidemia Panel

<table>
<thead>
<tr>
<th>BLOOD LIPIDS</th>
<th>LDL-C (mg/dL)</th>
<th>HDL-C (mg/dL)</th>
<th>Triglycerides (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial Hypercholesterolemia Panel, CATALOG NUMBER GBDNA2031</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABCA1</td>
<td>APOA2</td>
<td>APOB</td>
<td>EPHX2</td>
</tr>
<tr>
<td>Familial Hypertriglyceridemia Panel, CATALOG NUMBER GBDNA2032</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>APOA5</td>
<td>APOC2</td>
<td>APOE</td>
<td>GPR85BP1</td>
</tr>
</tbody>
</table>

Case Studies

Case 1:
2 family members with shared variants with one having an additional variant in ABCA6 that is known to increase cholesterol levels. Clinical data supports an additional cholesterol increasing effect.

Case 2:
3 family members with APOE4(--)FREIBURG. 2 of 3 family members also possessed a rare variant in ABCA7. Clinical data supports an additional cholesterol increasing effect.

Table 2. Genes analyzed by GBinsight Familial Hypercholesterolemia Panel

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Genotype</th>
<th>Cholesterol Levels</th>
</tr>
</thead>
</table>

Table 3. Genes analyzed by GBinsight Familial Hypertriglyceridemia Panel

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Genotype</th>
<th>Triglyceride Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>APOE4(--)FREIBURG</td>
<td>normal</td>
</tr>
<tr>
<td>21</td>
<td>APOE4(--)FREIBURG, ABCA7 p.Asp87Val (16:67977010-T-A)</td>
<td>+++</td>
</tr>
<tr>
<td>22</td>
<td>APOE4(--)FREIBURG, ABCA7 p.Asp87Val (16:67977010-T-A)</td>
<td>++</td>
</tr>
</tbody>
</table>
**Results**

Cohort consists of 55 patients from three US-based, academic preventive cardiology/lipid clinics with documented severe hypercholesterolemia (≥190mg/dL) sequenced with GBinsight Comprehensive Dyslipidemia panel (see Table 1 listing the genes sequenced and analyzed).

- Overall, we identified a cause for 78% of our cohort: 22 (40%) with a monogenic and 21 (38%) with a polygenic cause of their hypercholesterolemia. We did not identify a known cause for 12 (22%) patients.
- Each patient possessed a distinct mix of rare and common risk variants underscoring the heterogeneous nature of hypercholesterolemia and a CAD risk profile.

**Monogenic: FH**

- GBinsight identified 16 patients with one or more known familial hypercholesterolemia (FH) genetic variants (table 2).
  - 2 patients were compound heterozygotes: 1 patient with an LDLR/APOB and another with 2 LDLR missense variants.
  - Another LDLR variant was identified in 2 unrelated patients.

**Monogenic: APOE**

- We identified 6 patients with known pathogenic or suspected pathogenic variants in their APOE gene (table 2).
  - 1 patient was homozygous for the E2 allele, which is the most common cause of type III hyperlipoproteinemia.
  - 3 family members had a rare E4 variant – APOE4- FREIBURG.
  - A novel variant (p.Gln141Arg) that is predicted to be pathogenic due to it being located within a hotspot of other known pathogenic variants.

**High LP(a)**

- Very high LP(a) levels (≥50mg/dL) is an independent risk factor for ASCVD and aortic stenosis. High LP(a) is genetically determined by variants in the LPA gene and APOE. Nearly 1 in 5 people have high LP(a) and is more common in people of African and South Asian descent.
  - Of the 11 patients with confirmed very high LP(a) levels (>50 mg/dL) by clinical laboratory analysis, GBinsight detected a genetic variant known to cause elevated LP(a) in 6 of them: 1 each with p.Arg1421Gln and p.Thr1399Pro and 2 patients each with p.Ile1891Met and 6 with c.3947+467T>C (rs10455872).
  - 8/11 had at least one APOE-E4 allele, which is known to increase LP(a) levels.21
  - Another 11 patients were identified with one or more LP(a)-increasing variants for which confirmatory evidence was not available: 2 with p.Thr1399Pro, 3 with p.Ile1891Met and 6 with c.3947+467T>C (rs10455872).
  - In total, at least one LP(a)-increasing variant was identified in 17/55 (31%) patients.
  - GBinsight identified a very rare frameshift variant (p.Thr1901Alafs, rs74855355) in 2 members of a family that is likely protective against elevated LP(a).
Comprehensive genetic assessment of hypercholesterolemia and coronary artery disease risk

Polygenic hypercholesterolemia
An excess of individually modest risk variants can collectively cause hypercholesterolemia. GBinsight computes a polygenic score made up of rare and common variants in both coding and regulatory regions. Briefly, a per pathway (figure 2) GBscore is generated based on a weighted variant additive model that factors in both risk-increasing and protective variants. The GBscore is expressed on a 0-100 scale that computes a patient's pathway score relative to the population score for that patient (based on 1000Genomes Phase 3 allele frequencies.

- Of the 33 patients without an identified monogenic cause, 13 (39%) had a high (>75) polygenic score for hypercholesterolemia (see figure 3).
- Another 8 had a high (>75) polygenic score for combined hyperlipidemia (see figure 3).

Other pathways
In addition to hypercholesterolemia and elevated LP(a), other pathways contribute to an individual’s ASCVD risk.

- We identified 7 patients heterozygous for a known or predicted pathogenic variant in one of the genes in our panel that impact the RCT pathway (table 4).
- We identified 4 variants in APOA1, ABCA1 and LCAT (same variant in 2 related family members) that are predicted to affect RCT and further contribute to ASCVD.

- Mutations in ABCA1 or APOA1 significantly increase ASCVD risk even when HDL cholesterol levels are unchanged4,5.
- Figure 3 shows a heat map of all patients for all pathways scored.

Conclusions
1. Complex heterogeneity of dyslipidemias, even amongst those with monogenic causes has clinical implications.
2. Identifying known causes of disease is important as duration and amplitude of exposure to elevated lipids can profoundly affect risk.
3. Comprehensive analysis of the myriad of pathways converging on increased ASCVD risk may help with risk stratification and patient management.

References