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# THE PIVOTAL ROLE OF ABCB11 ON GENETIC SUSCEPTIBILITY TO DRUG-INDUCED LIVER INJURY FROM ANABOLIC STEROIDS


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DILIN Drug–Induced Liver Injury Network

## INTRODUCTION AND AIM

Anabolic steroids (AS) are commonly used by bodybuilders to enhance performance and muscle mass. Predisposed males can develop drug induced liver injury (DILI) characterized by prolonged and severe bland cholestasis with significant pruritus and elevated bilirubin with minimal GTT elevations.

We aimed to identify genetic variants associated with the risk of AS-induced bland cholestasis using whole genome sequence (WGS) analysis.

## METHOD

1. **Cohort**:
   93 males with AS-DILI enrolled in the DILIN prospective study were matched with 1012 ancestry-matched population controls from 1000 Genome Project (1KGP).

2. **Ancestry analysis:**
   Genetic ancestry was inferred by principal components (PCs) computed in EIGENSTRAT.

3. **Whole genome analysis**:
   *Gene/region-based analysis*: Rare (MAF < 0.01) damaging variants (REVEL, LOFTEE, SPLICEAI, METASVN) were analyzed by applying the SKAT-O test implemented in RVTEST[1] to aggregate their effect within each gene.
   Functionally annotated intergenic rare variants were analyzed by the STAAR-O method as implemented in STARTAApipeline[2] to aggregate their effect within the predicted/known regulatory regions associated with each gene.
   *Gene-set analysis (GSA)*: Explorative GSA was performed as implemented in RVTEST[1] to aggregate the effect of rare damaging variants (RDV) by relevant biological pathways. For this analysis, we selected 80 KEGG pathways with ABCB11 in the gene sets. To maximize the power of the study due to limited cases, we first examined 1) a list of 504 candidate genes and 2) any genes

## RESULTS

1. We identified 61 Non-Hispanic White (NHW) subjects; 12 Non-Hispanic Black (NHB) subjects; 16 Hispanic subjects; and 4 Asian subjects among cases with the following clinical features (**Table 1**) and matched them with 422 NHW, 321 NHB 169 Hispanics, and 100 Asians subjects.
2. Among the 504 genes selected to be associated with Anabolic Steroids metabolisms or cholestatic biological pathways, *ABCB11* was the only gene significantly enriched in RDV (P=7.7x10-6), passing the multiple correction threshold. The gene was still significant in an unrestricted analysis (**Figure 1A**). 9% of the cases carried at least one variant in the gene compared to >2% of population controls across different ancestry groups (**Table 2**).
3. RDVs were located in two functional domains of the ABCB11 protein (**Figure 2A**).
4. Cis-regulatory elements of *ABCB11* were only nominally enriched (CAGE-enhancer, P=0.03). Regulation of other candidate genes showed a stronger significance such as *GSK3B* (**Figure 1B**).
5. Gene-set analysis aggregating the effect of RDVs by biological pathway indicates that other transporter genes involved in the excretion of the bile acids and those genes involved in the regulation of *ABCB11* might play a role in the genetic susceptibility of AS-DILI (**Figure 2B**) due to their increased susceptibility.
6. In complex diseases, the genes harbouring pathogenic variants generally have a limited effect as typically carried by 5% or less of the diseased individuals. In contrast, *ABCB11* accounted for 9% of the AS-DILI risk (**Figure 2C**).

***Table 1*** *Clinical characteristics of the AS-DILI cohort*

| Feature GT | Number /Median | Proportion/Range |
|---|---|---|
| **Age** | 31.8 | 20.4 - 59.4 |
| **Weight (Kg)** | 87.9 | 50.5 - 136.1 |
| **Pre-existing conditions** | | |
| Diabetes/endocrine disorder | 2/93 | 2% |
| Alcohol use | 67/91 | 73.60% |
| HCV RNA positive | 6/83 | 6.50% |
| HIV Positive | 1/93 | 1.10% |
| Family history liver disease | 1/93 | 1.10% |
| **Latency (days)** | 54.0 | 2.0 - 737.0 |
| **Signs and symptoms at DILI onset** | | |
| Jaundice | 91/93 | 97.80% |
| Pruritus | 75/93 | 80.60% |
| Abdominal pain | 37/93 | 39.80% |
| Nausea | 53/93 | 57.00% |
| Rash | 18/93 | 19.40% |
| Fever | 10/93 | 10.80% |
| **Severity Score** | | |
| Mild (anicteric) | 0/93 | 0 |
| Moderate (icteric) | 26/93 | 28.00% |
| Moderate-hospitalized | 47/93 | 50.50% |
| Severe (organ failure) | 20/93 | 21.50% |
| Fatal | 0/93 | 0 |
| **R value at Presentation** | | |
| Cholestatic | 28/88 | 31.80% |
| Mixed | 32/88 | 36.40% |
| Hepatocellular | 28/88 | 31.80% |
| **Peak Values (median, Q1/Q3)** | | |
| GGT level (U/L) | 47.0 | 35.0, 69.0 |
| Total Bilirubin | 28.9 | 19.3 -39.7 |
| ALT | 198 | 140.0 - 368.0 |
| AST | 128 | 94.0 - 165.0 |
| Alk Phos (U/L) | 270 | 176. - 352.0 |

***Table 2*** *Carriage frequency of genetic variation in ABCB11 among cases and population controls across ethnic groups*

| | RDVs | | Regulatory elements | | Cumulative | |
|---|---|---|---|---|---|---|
| ***Ethnicity*** | ***CA*** | ***CTL*** | ***CA*** | ***CTL*** | ***CA*** | ***CTL*** |
| *NHW (61 vs 422)* | 7% | 2% | 3% | 0.005% | 10% | 2% |
| *NHB (16 vs 321)* | 8% | 2% | 0% | 5% | 8% | 6% |
| *Hispanics (16 vs 169)* | 13% | 1% | 0% | 6% | 13% | 7% |
| *Asians (4 vs 100 )* | 25% | 0% | 25% | | 50% | |
| **Totals** | **9%** | | **2%** | | **12%** | |

*RDV = rare damaging variants; CA = cases; CTL = controls; NWH = Non-Hispanics White; NHB = non-Hispanics Blacks.*

***Figure 1: Summary of the gene-based analysis in the main NHW cohort (61 AB-DILI vs 422 controls)***

(**A**) Manhattan plot of the genome-wide gene-based tests of rare damaging variants (RDVs). Each point represents a gene defined by the gene-based SKAT-O p-values and its genomic position for association with AS-DILI. Only genes with a cumulative minor allele count of >3 were included. The genes that reached the Bonferroni correction are highlighted in red. Diamonds are the genes belonging to the candidate gene list. (**B**) Manhattan plot of the candidate genome-wide regulatory elements based tests of rare variants. The plots show the p-values against their genomic position for each element associated with AS-DILI risk. Each point represents a p-value from STAAR-O test by element/region. The genes that reached the Bonferroni correction are highlighted in red. The shape of the point represents the type of regulatory elements as reported on the legend on the right.

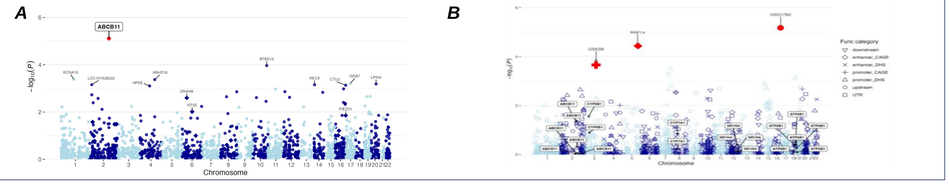
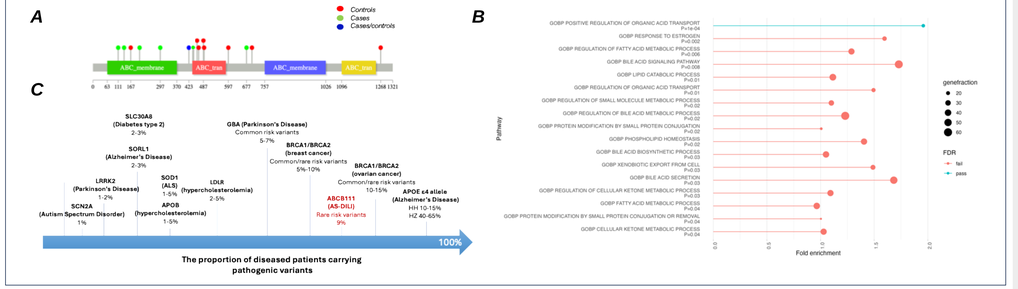



***Figure 2: Genomic location of the RDV in ABCB11 and its genetic relevance***

*Panel (**A**) shows the lollipop graph representing the location of the RDV within the protein domains. The colors of the dot represent the set of individuals carrying the variants (legend). Panel (**B**) The lollipop plot shows the significant (P<0.05) enrichment for KEGG pathways having ABCB11 in the gene-set. Panel (**C**) shows the proportion of diseased patients carrying known damaging/pathogenic variants (data extracted from the literature). For each gene, the disease and its frequency are reported. As shown in red, the ABCB11 gene has a relatively high proportion of AS-DILI cases carrying damaging variants compared with other disorders.*




## CONCLUSIONS

We identified a strong association between *ABCB11* gene and AS-DILI, with 9% of cases carrying at least one RDV across 4 different ethnic groups. As evidenced by the pathway analysis, other bile acids transporters/genes interacting or regulating ABCB11 function could also be involved in this genetic susceptibility. This study showed the feasibility of a WGS approach for a rare adverse event and supported the hypothesis that rare variants can lead to rare phenotypes with large effect sizes.

## REFERENCES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