Sex as a Biological Variable is Evident Following Deletion of Intestinal Apolipoprotein A1

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Background

- Apolipoprotein A1 (apoA1) is key to reverse cholesterol transport and HDL functionality, accounting for 70% of HDL protein mass[1]
- Human and mouse lipoprotein density profiles and apoproteins are similar[2,3]
- Whole body apoA1 knockout mice exhibit altered HDL composition and impaired HDL function[4]
- ApoA1 acts in innate immune function, neutralizing pathogens and bacterial antigens[5], and as an adaptor protein for MyD88[6]
- Given that 1/3 of HDL-C is lipidated by gut ABCA1[7], we wondered if intestinal apoA1 could play a critical role in dysfunctional HDL biogenesis.

Methods

Transgenic Mouse Construct

Single guide RNAs (sgRNAs, Sigma Millipore CRISPR Core) were used to insert LoxP sites flanking the largest exon of the ApoA1 gene (Figure 1) of C57BL/6 background mice. Correct insertion of LoxP sites was confirmed by PCR (1.3 kb band, Figure 2) followed by resequencing of the locus. Correct excision of the entire 4th exon of the ApoA1 gene following exposure to Cre recombinase under control of ubiquitous promoter (Tg(Sox2-cre)1 Amc/J) was also confirmed by PCR (330 bp band, Figure 3). ApoA1 floxed line founders were mated to Vil1-cre mice (B6.Cg-Tg(Vil1-cre)1000Gum/J, Jax Strain 021504) that express Cre recombinase in villus and crypt epithelial cells of the small and large intestines to produce mice that are heterozygous for tissue-specific knockout, so called ApoA1ifl/+ mice. Tissue-specific knockout mice (ApoA1 iKO) were confirmed by qPCR of tissues (Figure 4).

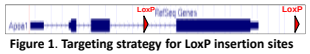


Figure 1. Targeting strategy for LoxP insertion sites

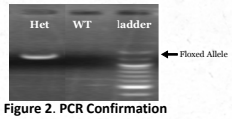


Figure 2. PCR Confirmation of Floxed Allele (1324 bp)

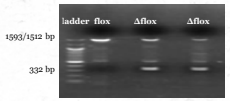


Figure 3. PCR Confirmation of Del Floxed Allele

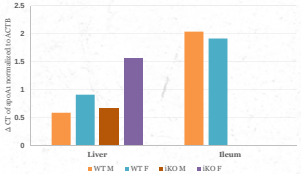


Figure 4. qPCR Confirmation of intestine-specific ApoA1 Deletion

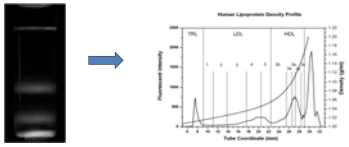
Animal Housing, Breeding, & Rearing

Animals were generated, reared, and housed at Texas A&M Institute for Genomic Medicine (TIGM) under standard 12:12-hour light:dark cycles. Sterilized, standard laboratory chow (4% Teklad Rodent Chow, Envigo) and water were provided *ad libitum*. 10- and 20-week-old mice were euthanized by CO_2 asphyxiation and exsanguinated by cardiac puncture. Data represent $apoA1^{fl/fl}$ (WT) males (WT M, n=9), $apoA1^{fl/fl}$ females (WT F, n=8), apoA1 intestinal knockout t males (iKO M, n=11), and apoA1 intestinal knockout females (iKO F, n=9).

Lipoprotein Profiling by Isopycnic Separation[8]

Circulating lipoproteins were stained by the fluorophore NBDC6-ceramide and then separated by NaBiEDTA density gradient ultracentrifugation. Pixel values of the center of the tube were converted into fluorescent intensity using Origin 8.0 software and plotted as a function of the tube coordinate (Figure 5). Lipoprotein subclasses were identified by their density ranges and total mass was quantified by measuring the area under the curve.

Figure 5. Representative Human Lipoprotein Density Profile Generated by 0.18M NaBiEDTA density gradient ultracentrifugation



Statistics

Estimates of differences between genotypes were determined by ANOVA and Tukey's HSD of mean values. Values are expressed as means +/- standard error. Differing letters denote significance at p<0.05. Lipoprotein data for heterozygous males are not included in statistical analysis and are included for reference only.

Results & Discussion

Figure 5. Organs (%BW)

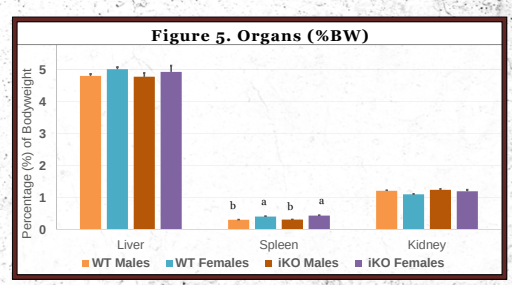


Table 1. Body and Organ Weights (g)

	WT Males n=9	WT Females n=8	iKO Males n=11	iKO Females n=9
Bodyweight	32.4 ± 0.74[a]	23.0 ± 0.69[b]	31.0 ± 0.68[a]	22.8 ± 0.99[b]
Liver	1.47 ± 0.06[a]	1.10 ± 0.04[b]	1.46 ± 0.04[a]	1.07 ± 0.03[b]
Spleen	0.09 ± 0.00	0.10 ± 0.00	0.09 ± 0.00	0.10 ± 0.00
Kidney	0.37 ± 0.01[a]	0.25 ± 0.01[b]	0.38 ± 0.01[a]	0.26 ± 0.00[b]

- Mice grew and bred normally, following growth curves for C57Bl6 mice[9]
- Males were generally heavier than females, but did not differ by genotype (p<0.0001)
- Spleens, as %BW, were larger (0.03% vs 0.04%) in females vs males, regardless of genotype (p<0.0001)

Figure 6. Lipoprotein Density Classes

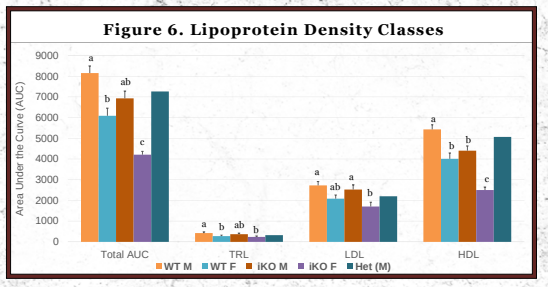


Figure 8. Lipoprotein Density Profiles

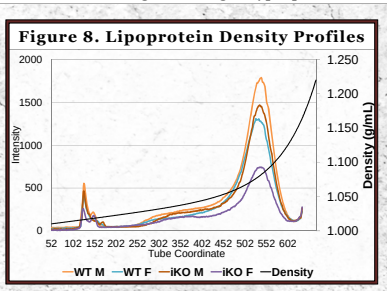
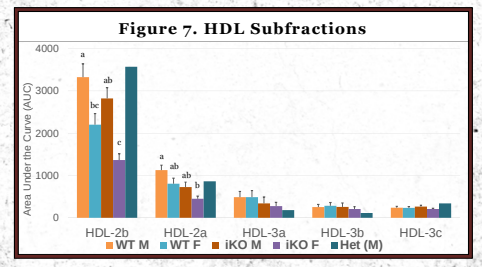


Figure 7. HDL Subfractions



- iKO reduced total lipoprotein mass by 28% in iKO F and 23% in iKO M compared to WT (p<0.0006)
- Total HDL (AUC) was lowest in iKO F, being reduced 38% in iKO F compared to WT F and 60% compared to M of either genotype (p<0.0001)
- HDL-2b was most reduced in iKO F, decreasing 39% vs. WT F, 52% vs. iKO M and 59% vs. WT M (p<0.02 for all)
- No differences were observed in HDL-3 subfractions (p<0.32)

Conclusions & Future Directions

- Intestinal apoA1 deletion results in loss of lipoprotein mass (assessed by AUC) in both genders with greatest effect in females
- Females significantly reduced total HDL, specifically HDL-2b and HDL-2a subfractions.
- The extent to which sex differences in intestinal apoA1 biology influences other sex-linked differences in health or disease development warrants further study.

References

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