Genetic Variants and Dyslipidemia

Dr. Kari North

12 December, 2016

Introduction

Dyslipidemia in children

- Dyslipidemia is an important risk factor for chronic cardiometabolic diseases.
- Dsylipidemia represents any abnormal levels of lipid, lipoprotein, or apolipoprotein factors (Kwiterovich 2008), including:
  - *total cholesterol (TC)*
  - *low density lipoprotein cholesterol (LDL-C)*
  - *triglycerides (TG)*
  - *high density lipoprotein choleseterol (HDL-C)*

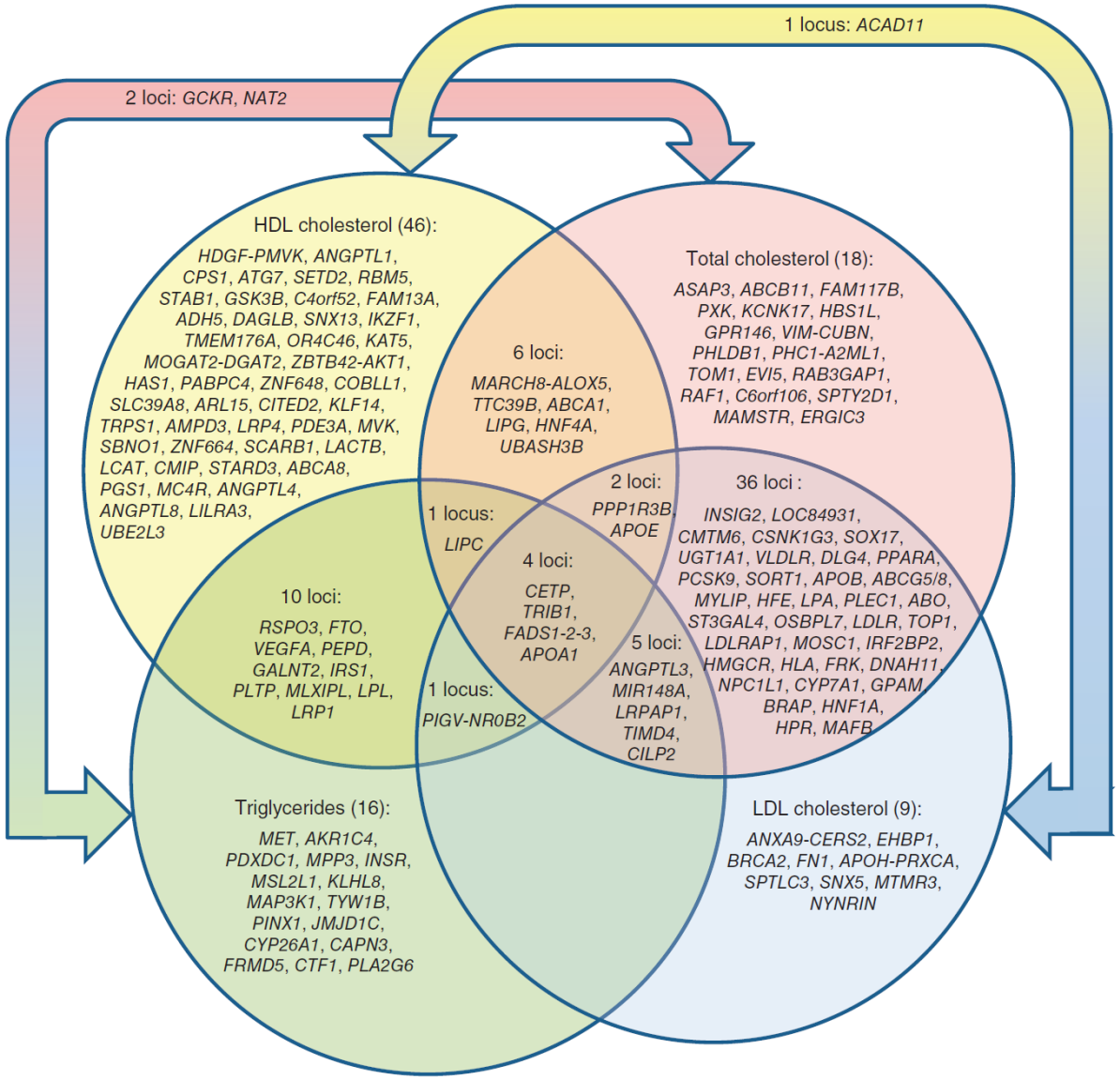
Introduction

Heritability of dyslipidemia

- Lipid traits are heritable (h^2 can exceed 0.50) (Goode et al. 2007) and strongly associated with cardiovascular disease sustaining strong interest in genetic research.
- By 2013, researchers have identified more than 150 established loci influencing lipid levels in adults (C. J. Willer et al. 2013).

Introduction

Lipid variants



Source: Willer, C. J., Schmidt, E. M., Sengupta, S., Peloso, G. M., Gustafsson, S., Kanoni, S., Abecasis, G. R. (2013). Discovery and refinement of loci associated with lipid levels. Nature Genetics, 45(11), 1274–1283.

https://doi.org/10.1038/ng.2797

Introduction

Generalizability across ancestral groups

- Results found for adults have been found to generalize to children in a Finnish population (Tikkanen et al. 2011; Buscot et al. 2016).

Association of Known Loci With Lipid Levels Among Children and Prediction of Dyslipidemia in Adults

Emmi Tikkanen, MSc*; Tarja Tuovinen, MSc*; Elisabeth Widén, MD, PhD; Terho Lehtimäki, MD, PhD; Jorma Viikari, MD, PhD; Mika Kähönen, MD, PhD; Leena Peltonen, MD, PhD†; Olli T. Raitakari, MD, PhD; Samuli Ripatti, PhD

- Certain variants that associate with a trait such as dyslipdemia in one ancestral group may not associate in other groups (Carlson et al. 2013).
  - *Why? Differences across ancestral groups include:*
    - Variant frequencies
    - Linkage disequilibrium
- No research exists examining the association between genetic variants and dyslipidemia in Hispanic/Latino (HL) children

Introduction

Primary Aim

- Examine the association of known lipid variants with lipid traits identified in large study of adult participants from a Chilean infancy cohort of primarily European-descent.
    - *Compare results to those found in a Finnish population.*

Methods

Association tests

- We assessed single variant associations using linear regression for high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglycerides (TG), assuming an additive genetic model, adjusted for sex and ancestry (via principal components).

- Sample Model for HDL
  - $HDL_i = \beta_0 + SNP_i\beta_1 + sex_i\beta_2 + ANCESTRY_i\boldsymbol{\beta} + \epsilon_i$
  - SNP_i *represents one SNP with "genotypes were coded as 0, 1, or 2 when directly genotyped or as a predicted allele dosage (range, 0–2) when imputed." (Tikkanen et al. 2011)*

- Only six variants from the Chilean sample met the *a priori* threshold of power > 0.8 to detect an association based on effect sizes from GWAS (T. M. Teslovich et al. 2010).

Methods

Polygenic risk scores

- Regress phenotypes onto weighted trait-specific polygenic risk scores (PRS).
- Sample model for HDL
  - $HDL_i = \beta_0 + \beta_1 PRS1_i + \epsilon_i$
  - *PRS for HDL-C*
    - (0.48 * rs4660293 + 0.49 * rs2814944 + 0.59 * rs4731702 + 0.41 * rs2923084 + 0.4 * rs7134375 + 0.44 * rs7134594 + 1.45 * rs1532085 + 3.39 * rs3764261 + 0.45 * rs2925979 + 0.42 * rs4148008 + 0.39 * rs4129767 + 0.64 * rs737337 + 1.88 * rs1800961 + 0.93 * rs6065906 + 0.47 * rs1689800 + 0.61 * rs4846914 + 0.68 * rs12328675 + 0.46 * rs2972146 + 0.49 * rs6450176 + 0.39 * rs605066 + 1.95 * rs1084651 + 1.21 * rs9987289 + 0.44 * rs2293889 + 0.65 * rs581080 + 0.94 * rs1883025 + 0.78 * rs3136441 + 0.86 * rs4759375 + 0.44 * rs4765127 + 0.61 * rs838880 + 0.39 * rs2652834 + 1.27 * rs16942887 + 0.48 * rs11869286 + 1.31 * rs7241918 + 0.42 * rs12967135 + 0.45 * rs7255436 + 0.83 * rs386000 + 0.46 * rs181362 + 0.84 * rs13107325) / 29.79
- All PRS are standardized in the regression models: regression coefficient for PRS indicates a one unit change in SD of PRS.

Methods

Proportion of variance explained by lipid-related SNPs

- Linear models containing all SNPs related to a specific phenotype, such as HDL-C, will be covariates.
- The continuous lipid phenotype is the outcome.
- Differences in R^2 will be calculated between models with and without the SNPs to estimate h^2.
  - *Model for HDL:*
    - HDL_i = b0 + b1 * rs4660293 + b2 * rs2814944 + b3 * rs4731702 + b4 * rs2923084 + b5 * rs7134375 + b6 * rs7134594 + b7 * rs1532085 + b8 * rs3764261 + b9 * rs2925979 + b10 * rs4148008 + b11 * rs4129767 + b12 * rs737337 + b13 * rs1800961 + b14 * rs6065906 + b15 * rs1689800 + b16 * rs4846914 + b17 * rs12328675 + b18 * rs2972146 + b19 * rs6450176 + b20 * rs605066 + b21 * rs1084651 + b22 * rs9987289 + b23 * rs2293889 + b24 * rs581080 + b25 * rs1883025 + b26 * rs3136441 + b27 * rs4759375 + b28 * rs4765127 + b29 * rs838880 + b30 * rs2652834 + b31 * rs16942887 + b32 * rs11869286 + b33 * rs7241918 + b34 * rs12967135 + b35 * rs7255436 + b36 * rs386000 + b37 * rs181362 + b38 * rs13107325 + ϵ_i

Results

Descriptive statistics

Descriptive statistics by gender, median (interquartile range)

Trait	Female (n=263)	Male (n=283)
log(TG (mmol/l))	1.44 (0.53)	1.38 (0.6)
LDL-C (mmol/l)	5.26 (1.55)	5.02 (1.53)
HDL-C (mmol/l)	2.3 (0.77)	2.05 (0.66)
TC (mmol/l)	8.55 (1.79)	7.96 (1.65)
Age (years)	16.77 (0.3)	16.76 (0.31)
BMI (kg/m2)	23.25 (5.33)	22.31 (5.12)

- Note: All subsequent references to TG refer to log(TG).

Results

Explained variance

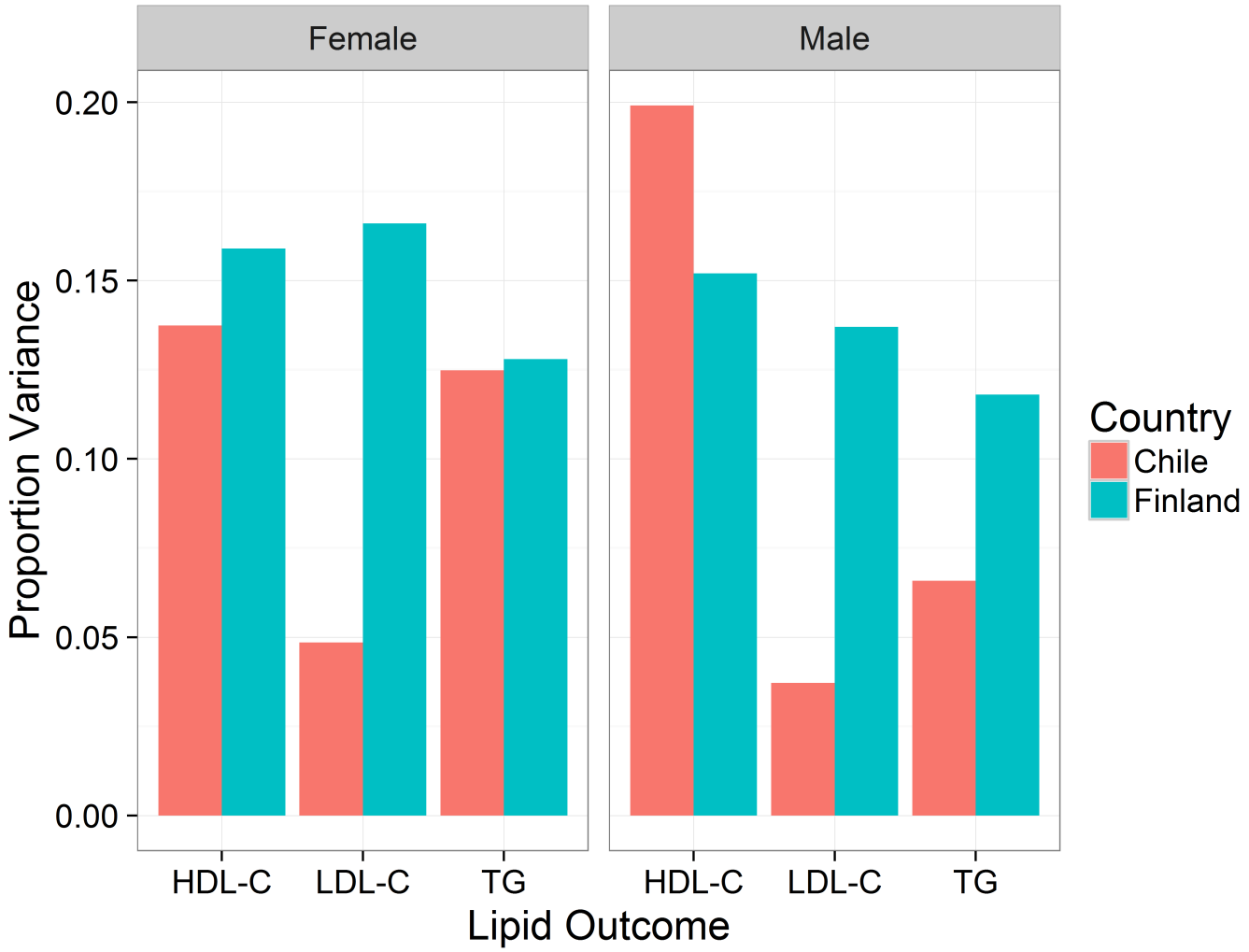
Proportion of lipid traits variance explained by lipid-related variants, by gender

Gender	HDL-C	LDL-C	TG
female	0.137	0.049	0.125
male	0.199	0.037	0.066

- The lipid loci explained the least amount of total variance for LDL (males=4% and females=5%) and the most amount of variance for HDL (males=20% and females=14%).

Results

Explained variance by gender and country



Results

Candidate single variant tests of association

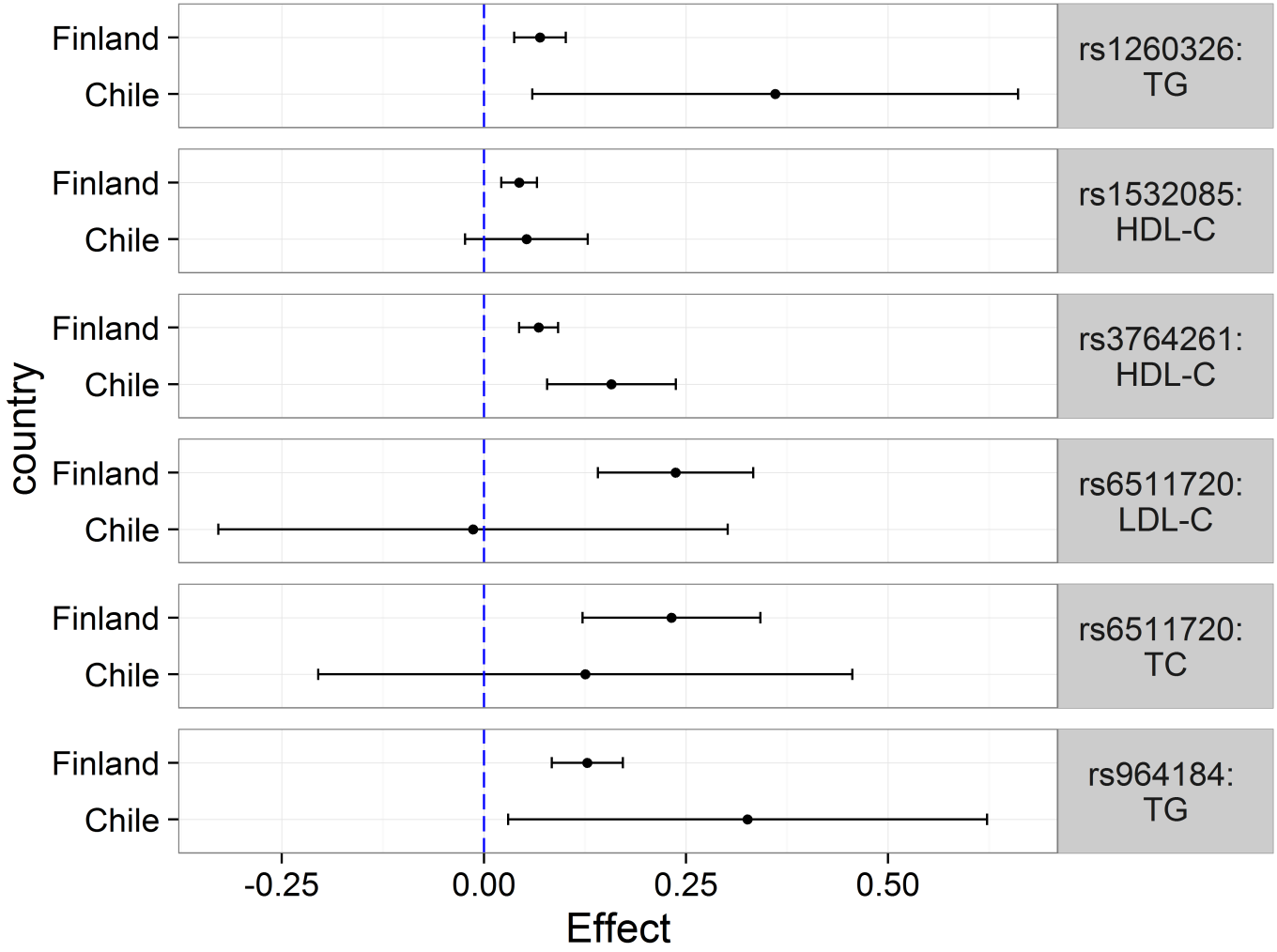
Association between single variants and and serum lipid levels, additive model

rsid	Trait	Ref/Min allele	MAF	Effect (se)	p-value
rs1532085	HDL-C	G/A	0.33	0.053 (0.038)	0.1655
rs3764261	HDL-C	C/A	0.31	0.158 (0.04)	1e-04
rs6511720	LDL-C	G/T	0.07	-0.013 (0.158)	0.9318
rs1260326	TG	C/T	0.31	0.36 (0.15)	0.0165
rs964184	TG	C/G	0.37	0.326 (0.148)	0.0277
rs6511720	TC	G/T	0.07	0.125 (0.165)	0.4481

- For each significant variant, direction of effect matched the multiethnic adult GWAS from which SNPs were selected.

Results

Candidate single variant tests of association by variant and country



Note: Adjusted for sex of child and first five principal components representing ancestry.

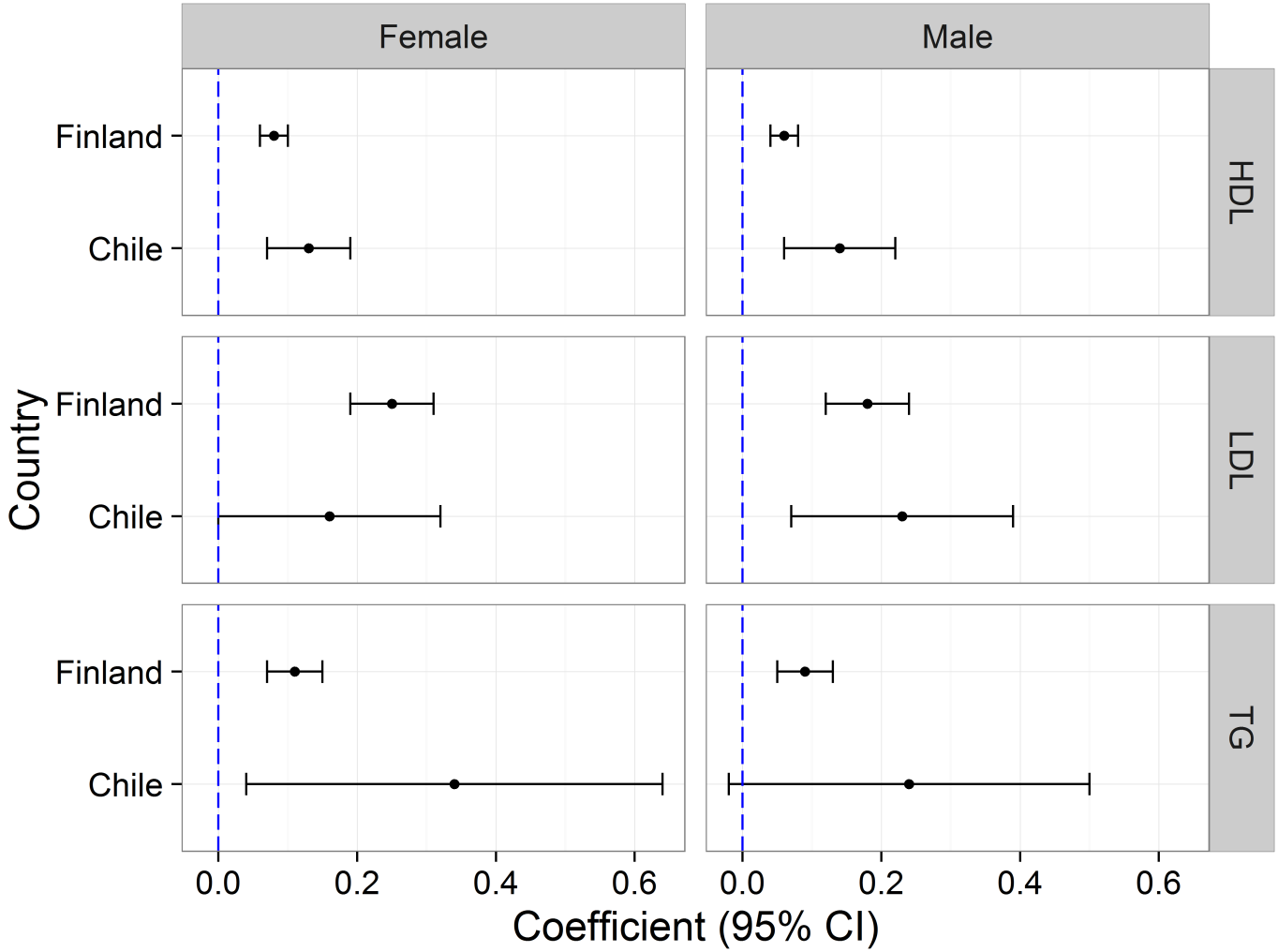
Results: Weighted polygenic risk score (wPRS) regression coefficients

Association between the genetic risk score and serum lipid levels, coefficient (SE)

	Female		Male	
Outcome/trait	**Not adjusted**	**Adjusted for BMI**	**Not adjusted**	**Adjusted for BMI**
HDL	0.13 (0.03)	0.13 (0.03)	0.14 (0.04)	0.14 (0.04)
LDL	0.16 (0.08)	0.17 (0.08)	0.23 (0.08)	0.24 (0.08)
TG	0.34 (0.15)	0.08 (0.02)	0.24 (0.13)	0.05 (0.02)

- All estimates significant at α level of 0.05 with the exception of TG levels for males.

Results: Weighted polygenic risk score (wPRS) regression coefficients by country and gender



Notes: Coefficients represent change in outcome per 1 SD change in wGRS, adjusted for first five principal components representing ancestry but NOT BMI.

Conclusion

Evidence for associations

- There is evidence that lipid loci from a HL sample of adolescents contain similar associations as those from European children and adults.
- Despite the small sample size and possibility for bias with different ancestral groups we found meaningful and statistically significant associations relating lipid loci in a HL cohort of Chilean adolescents with those found in European ancestral groups.
- These associations emphasize the importance of adolescence as a time for disease prevention given studies demonstrating both the persistence of associations between PRS and lipids over the life course and the increasing role PRS plays in predicting disease.

Extra slides, estimates from Tikkanen et al. (Tikkanen et al. 2011)

Descriptive statistics

Descriptive statistics

Outcome/Trait	Female	Male
N	661	555
TG (log(mmol/l))	0.90 (0.37)	0.91 (0.39)
LDL-C (mmol/l)	3.07 (0.79)	2.91 (0.79)
HDL-C (mmol/l)	1.55 (0.29)	1.34 (0.24)
TC (mmol/l)	5.02 (0.89)	4.67 (0.84)

Extra slides, estimates from Tikkanen et al. (Tikkanen et al. 2011)

Proportion variance explained by lipid variants

Proportion of lipid traits variance explained by lipid-related variants, by gender

Gender	HDL-C	LDL-C	TG
Female	0.159	0.166	0.128
Male	0.152	0.137	0.118

Extra slides, estimates from Tikkanen et al. (Tikkanen et al. 2011)

Single variant association tests

Association between the genetic risk score and serum lipid levels, coefficient (SE)

rs id	Trait	Effect (se)	Locus	p-value
rs3764261	HDL-C	0.0676 (0.012)	CETP	1.5e-08
rs1532085	HDL-C	0.0435 (0.011)	LIPC	6.9e-05
rs6511720	LDL-C	0.2370 (0.048)	LDLR	7.1e-07
rs1260326	TG	0.0693 (0.016)	GCKR	1.7e-05
rs964184	TG	0.1278 (0.022)	APOA1-C3-A4-A5	5.8e-09
rs6511720	TC	0.2320 (0.055)	LDLR	2.2e-05

Extra slides, estimates from Tikkanen et al. (Tikkanen et al. 2011)

Polygenic risk score

Association between the genetic risk score and serum lipid levels, coefficient (SE)

Outcome/Trait	Female	Male
HDL	0.08 (0.01)	0.06 (0.01)
LDL	0.25 (0.03)	0.18 (0.03)
TG	0.11 (0.02)	0.09 (0.02)

Extra slides: effect sizes used in PRS from Teslovich et al. 2010 (T. M. Teslovich et al. 2010).

Locus	Chr	Lead SNP	Lead trait	Other traits	Alleles/MAF	Effect size
LDLRAP1	1	rs12027135	TC	LDL	T/A/0.45	-1.22
PABPC4	1	rs4660293	HDL		A/G/0.23	-0.48
PCSK9	1	rs2479409	LDL	TC	A/G/0.30	2.01
ANGPTL3	1	rs2131925	TG	TC, LDL	T/G/0.32	-4.94
EVI5	1	rs7515577	TC		A/C/0.21	-1.18
SORT1	1	rs629301	LDL	TC	T/G/0.22	-5.65
ZNF648	1	rs1689800	HDL		A/G/0.35	-0.47
MOSC1	1	rs2642442	TC	LDL	T/C/0.32	-1.39
GALNT2	1	rs4846914	HDL	TG	A/G/0.40	-0.61
IRF2BP2	1	rs514230	TC	LDL	T/A/0.48	-1.36
APOB	2	rs1367117	LDL	TC	G/A/0.30	4.05
	NA	rs1042034	TG	HDL	T/C/0.22	-5.99
GCKR	2	rs1260326	TG	TC	C/T/0.41	8.76
ABCG5/8	2	rs4299376	LDL	TC	T/G/0.30	2.75
RAB3GAP1	2	rs7570971	TC		C/A/0.34	1.25
COBLL1	2	rs10195252	TG		T/C/0.40	-2.01
	NA	rs12328675	HDL		T/C/0.13	0.68
IRS1	2	rs2972146	HDL	TG	T/G/0.37	0.46
RAF1	3	rs2290159	TC		G/C/0.22	-1.42
MSL2L1	3	rs645040	TG		T/G/0.22	-2.22

Locus	Chr	Lead SNP	Lead trait	Other traits	Alleles/MAF	Effect size
KLHL8	4	rs442177	TG		T/G/0.41	-2.25
SLC39A8	4	rs13107325	HDL		C/T/0.07	-0.84
ARL15	5	rs6450176	HDL		G/A/0.26	-0.49
MAP3K1	5	rs9686661	TG		C/T/0.20	2.57
HMGCR	5	rs12916	TC	LDL	T/C/0.39	2.84
TIMD4	5	rs6882076	TC	LDL, TG	C/T/0.35	-1.98
MYLIP	6	rs3757354	LDL	TC	C/T/0.22	-1.43
HFE	6	rs1800562	LDL	TC	G/A/0.06	-2.22
HLA	6	rs3177928	TC	LDL	G/A/0.16	2.31
	NA	rs2247056	TG		C/T/0.25	-2.99
C6orf106	6	rs2814944	HDL		G/A/0.16	-0.49
	NA	rs2814982	TC		C/T/0.11	-1.86
FRK	6	rs9488822	TC	LDL	A/T/0.35	-1.18
CITED2	6	rs605066	HDL		T/C/0.42	-0.39
LPA	6	rs1564348	LDL	TC	T/C/0.17	-0.56
	NA	rs1084651	HDL		G/A/0.16	1.95
DNAH11	7	rs12670798	TC	LDL	T/C/0.23	1.43
NPC1L1	7	rs2072183	TC	LDL	G/C/0.25	2.01
TYW1B	7	rs13238203	TG		C/T/0.04	-7.91
MLXIPL	7	rs17145738	TG	HDL	C/T/0.12	-9.32
KLF14	7	rs4731702	HDL		C/T/0.48	0.59
PPP1R3B	8	rs9987289	HDL	TC, LDL	G/A/0.09	-1.21
PINX1	8	rs11776767	TG		G/C/0.37	2.01

Locus	Chr	Lead SNP	Lead trait	Other traits	Alleles/MAF	Effect size
NAT2	8	rs1495741	TG	TC	A/G/0.22	2.85
LPL	8	rs12678919	TG	HDL	A/G/0.12	-13.64
CYP7A1	8	rs2081687	TC	LDL	C/T/0.35	1.23
TRPS1	8	rs2293889	HDL		G/T/0.41	-0.44
	NA	rs2737229	TC		A/C/0.30	-1.11
TRIB1	8	rs2954029	TG	TC, LDL, HDL	A/T/0.47	-5.64
PLEC1	8	rs1113634 1	LDL	TC	A/G/0.40	11.40
TTC39B	9	rs581080	HDL	TC	C/G/0.18	-0.65
ABCA1	9	rs1883025	HDL	TC	C/T/0.25	-0.94
ABO	9	rs9411489	LDL	TC	C/T/0.20	2.24
JMJD1C	10	rs10761731	TG		A/T/0.43	-2.38
CYP26A1	10	rs2068888	TG		G/A/0.46	-2.28
GPAM	10	rs2255141	TC	LDL	G/A/0.30	1.14
AMPD3	11	rs2923084	HDL		A/G/0.17	-0.41
SPTY2D1	11	rs10128711	TC		C/T/0.28	-1.04
LRP4	11	rs3136441	HDL		T/C/0.15	0.78
FADS1-2-3	11	rs174546	TG	HDL, TC, LDL	C/T/0.34	3.82
APOA1	11	rs964184	TG	TC, HDL, LDL	C/G/0.13	16.95
UBASH3B	11	rs7941030	TC	HDL	T/C/0.38	0.97
ST3GAL4	11	rs11220462	LDL	TC	G/A/0.14	1.95
PDE3A	12	rs7134375	HDL		C/A/0.42	10.40
LRP1	12	rs11613352	TG	HDL	C/T/0.23	22.70
MVK	12	rs7134594	HDL		T/C/0.47	-0.44

Locus	Chr	Lead SNP	Lead trait	Other traits	Alleles/MAF	Effect size
BRAP	12	rs11065987	TC	LDL	A/G/0.42	-0.96
HNF1A	12	rs1169288	TC	LDL	A/C/0.33	1.42
SBNO1	12	rs4759375	HDL		C/T/0.06	0.86
ZNF664	12	rs4765127	HDL	TG	G/T/0.34	0.44
SCARB1	12	rs838880	HDL		T/C/0.31	0.61
NYNRIN	14	rs8017377	LDL		G/A/0.47	1.14
CAPN3	15	rs2412710	TG		G/A/0.02	7.00
FRMD5	15	rs2929282	TG		A/T/0.05	5.13
LIPC	15	rs1532085	HDL	TC, TG	G/A/0.39	1.45
LACTB	15	rs2652834	HDL		G/A/0.20	-0.39
CTF1	16	rs11649653	TG		C/G/0.40	-2.13
CETP	16	rs3764261	HDL	TC, LDL, TG	C/A/0.32	3.39
LCAT	16	rs16942887	HDL		G/A/0.12	1.27
HPR	16	rs2000999	TC	LDL	G/A/0.20	2.34
CMIP	16	rs2925979	HDL		C/T/0.30	-0.45
STARD3	17	rs11869286	HDL		C/G/0.34	-0.48
OSBPL7	17	rs7206971	LDL	TC	G/A/0.49	0.78
ABCA8	17	rs4148008	HDL		C/G/0.32	-0.42
PGS1	17	rs4129767	HDL		A/G/0.49	-0.39
LIPG	18	rs7241918	HDL	TC	T/G/0.17	-1.31
MC4R	18	rs12967135	HDL		G/A/0.23	-0.42
ANGPTL4	19	rs7255436	HDL		A/C/0.47	-0.45
LDLR	19	rs6511720	LDL	TC	G/T/0.11	-6.99

Locus	Chr	Lead SNP	Lead trait	Other traits	Alleles/MAF	Effect size
LOC55908	19	rs737337	HDL		T/C/0.08	-0.64
CILP2	19	rs10401969	TC	TG, LDL	T/C/0.07	-4.74
APOE	19	rs4420638	LDL	TC, HDL	A/G/0.17	7.14
	NA	rs439401	TG		C/T/0.36	25.50
FLJ36070	19	rs492602	TC		A/G/0.49	1.27
LILRA3	19	rs386000	HDL		G/C/0.20	0.83
ERGIC3	20	rs2277862	TC		C/T/0.15	-1.19
MAFB	20	rs2902940	TC	LDL	A/G/0.29	-1.38
TOP1	20	rs6029526	LDL	TC	T/A/0.47	1.39
HNF4A	20	rs1800961	HDL	TC	C/T/0.03	-1.88
PLTP	20	rs6065906	HDL	TG	T/C/0.18	-0.93
UBE2L3	22	rs181362	HDL		C/T/0.20	-0.46
PLA2G6	22	rs5756931	TG		T/C/0.40	-1.54

Extra slides: Other work

1. AHA EPI/Lifestyle 2015 Poster: Childhood body mass index (BMI) z-scores associated with low HDL-C levels in adolescence in a Chilean cohort

2. UNC EPID 726 class presentation in advance of AHA predoctoral (successful) grant application (2016-2018)

3. Questions about analyses? Please visit the github web page!

4. Assistance with slides by Ann Von Holle, doctoral student

References

Buscot, Marie-jeanne, Costan G. Magnussen, Markus Juonala, Niina Pitkänen, Terho Lehtimäki, Jorma S. A. Viikari, Mika Kähönen, et al. 2016. "The Combined Effect of Common Genetic Risk Variants on Circulating Lipoproteins Is Evident in Childhood: A Longitudinal Analysis of the Cardiovascular Risk in Young Finns Study." Edited by David Fardo. *PLOS ONE* 11 (1): e0146081. doi:10.1371/journal.pone.0146081.

Carlson, Christopher S., Tara C. Matise, Kari E. North, Christopher A. Haiman, Megan D. Fesinmeyer, Steven Buyske, Fredrick R. Schumacher, et al. 2013. "Generalization and Dilution of Association Results from European GWAS in Populations of Non-European Ancestry: The PAGE Study." Edited by Greg Gibson. *PLoS Biology* 11 (9): e1001661. doi:10.1371/journal.pbio.1001661.

Goode, Ellen L., Stacey S. Cherny, Joe C. Christian, Gail P. Jarvik, and Mariza de Andrade. 2007. "Heritability of Longitudinal Measures of Body Mass Index and Lipid and Lipoprotein Levels in Aging Twins." *Twin Research and Human Genetics* 10 (5): 703–11. doi:10.1375/twin.10.5.703.

Kwiterovich, Peter O. 2008. "Clinical and Laboratory Assessment of Cardiovascular Risk in Children: Guidelines for Screening, Evaluation, and Treatment." *Journal of Clinical Lipidology* 2 (4): 248–66. doi:10.1016/j.jacl.2008.06.003.

Teslovich, Tanya M., Kiran Musunuru, Albert V. Smith, Andrew C. Edmondson, Ioannis M. Stylianou, Masahiro Koseki, James P. Pirruccello, et al. 2010. "Biological, Clinical and Population Relevance of 95 Loci for Blood Lipids." *Nature* 466 (7307): 707–13. doi:10.1038/nature09270.

Tikkanen, E., T. Tuovinen, E. Widen, T. Lehtimaki, J. Viikari, M. Kahonen, L. Peltonen, O. T. Raitakari, and S. Ripatti. 2011. "Association of Known Loci with Lipid Levels Among Children and Prediction of Dyslipidemia in Adults." *Circulation: Cardiovascular Genetics* 4 (6): 673–80. doi:10.1161/CIRCGENETICS.111.960369.

Willer, Cristen J, Ellen M Schmidt, Sebanti Sengupta, Gina M Peloso, Stefan Gustafsson, Stavroula Kanoni, Andrea Ganna, et al. 2013. "Discovery and Refinement of Loci Associated with Lipid Levels." *Nature Genetics* 45 (11): 1274–83. doi:10.1038/ng.2797.