Protein-altering variants (PAV) associated with BMI in the general population and their roles in survivors of childhood cancer

Working group: Genetics

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Background:

Survivors of childhood cancer are at increased risk of obesity[1] – an important risk factor for many chronic diseases (hypertension, type 2 diabetes, cardiovascular disease and cancer) associated with morbidity and premature mortality. Risk factors for obesity among survivors include exposure to cranial radiation therapy (CRT), corticosteroids, reduced physical activity, energy expenditure during exercise and childhood obesity[2-5]. However, these factors alone do not fully explain the inter-individual variability in risk of obesity among survivors of childhood cancer, suggesting a role of genetic susceptibility. However, little is known about genetic factors associated with BMI/obesity in survivors of childhood cancer.

Genome-wide association (GWA) studies in the general population[6] have identified ~1000 common SNPs associated with BMI, which have provided important information about underlying molecular mechanisms of the trait. Recently, a large GWA study was conducted by the GIANT consortium[7], including >700,000 individuals from across the world, which robustly implicated 14 rare and low-frequency coding variants in BMI [see below Table 1 from Turcot et al. *Nature Genetics* (2018)]. These variants have, on average, >10-fold greater effect on BMI compared to the common BMI loci. The largest effect was for a rare variant on *MC4R* which was associated with ~7 kg increase in body weight for a 1.7-meter tall person. Many of these BMI-associated variants were also associated with cardiometabolic traits. The BMI increasing allele of the *ZBTB7B* SNV encoding p.Pro190Ser was associated with greater height, and those for the *PRKAG1*, *ACHE* and *RAPGEF3* SNVs were associated with shorter height, but association with other traits differed. Specifically, carriers of the serine-encoding allele for *PRKAG1* p.Thr38Ser appeared heavier and shorter and had lower HDL cholesterol levels, earlier age at menarche. While carriers of the proline-encoding allele for *RAPGEF3* p.Leu300Pro were also heavier and shorter, they had a lower BMI-adjusted waist-to-hip ratio (WHRadjBMI) and lower fasting

insulin levels. Thus, while all SNVs were associated with BMI, their patterns of association with other traits suggest that they may affect different physiological pathways.

Table 1 | Rare and low-frequency coding variants significantly associated with BMI

Chr:position	Variant	Coding locus	Allele		Amino acid change	EAF (%)	β (s.d./ allele)	SE	P value	N	Explained variance (%)
			Effect	Other							
All ancestry additive											
1:154987704	rs141845046	*ZBTB7B*[a]	T	C	p.Pro190Ser	2.44	0.048	0.006	7.73×10^{-18}	718,628	0.011
7:100490797	rs1799805	*ACHE*[a]	T	G	p.His353Asn	3.90	0.029	0.005	2.82×10^{-10}	707,448	0.006
12:48143315	rs145878042	*RAPGEF3*[a]	G	A	p.Leu300Pro	1.10	0.066	0.008	1.56×10^{-15}	700,852	0.010
12:49399132	rs1126930	*PRKAG1*	C	G	p.Thr38Ser	3.22	0.034	0.005	3.98×10^{-12}	712,354	0.007
12:72179446	rs61754230	*RAB21*[a]	T	C	p.Ser224Phe	1.74	0.040	0.007	1.33×10^{-9}	693,373	0.005
12:117977550	rs56214831	*KSR2*	T	C	p.Arg525Gln	0.82	0.057	0.010	1.08×10^{-8}	655,049	0.005
12:123345509	rs34149579	*HIP1R*	T	G	p.Cys938Phe	4.54	−0.032	0.004	2.00×10^{-14}	716,253	0.009
16:72830539	rs62051555	*ZFHX3*[a]	G	C	p.Gln1100His	4.34	−0.024	0.004	4.01×10^{-8}	690,637	0.005
18:58039478	rs13447324	*MC4R*	T	G	p.Tyr35Ter	0.01	0.542	0.086	2.26×10^{-10}	631,683	0.006
19:46178020	rs139215588	*GIPR*	A	G	p.Arg190Gln	0.11	−0.148	0.028	1.25×10^{-7}	695,800	0.005
19:46180976	rs143430880	*GIPR*	G	A	p.Glu288Gly	0.13	−0.153	0.028	2.96×10^{-8}	599,574	0.006
20:25195509	rs6050446	*ENTPD6*[a]	A	G	p.Lys185Glu	2.71	−0.034	0.005	2.40×10^{-10}	717,084	0.006
All ancestry sex specific additive (women only)											
19:3813906	rs45465594	*ZFR2*[a]	C	A	p.Ile718Met	2.55	−0.040	0.008	1.94×10^{-7}	373,848	0.008
European ancestry only additive											
9:97062981	rs12236219	*ZNF169*[a]	T	C	p.Arg381Cys	4.23	−0.029	0.005	8.78×10^{-10}	612,396	0.007

Array-wide significance is defined as $P < 2 \times 10^{-7}$. Variant positions are reported according to Build 37, and alleles are coded according to the positive strand. Alleles (effect/other), effect allele frequency (EAF), β value, standard error (SE) and P value are based on the meta-analysis of discovery-stage (GIANT) and validation-stage (deCODE, UK Biobank) studies. The effect allele is always the minor allele. Effects (β) are expressed in s.d., assuming mean = 0 and s.d. = 1. The amino acid change from the most abundant coding transcript is shown in this table (see Supplementary Table 24 for more details on protein annotation based on the VEP tool and transcript abundance from the GTEx database).[a] New gene (not previously implicated in human obesity).

Herein, we propose to assess these 14 rare and low-frequency coding variants for their associations with BMI/obesity in survivors of childhood cancer. Specifically, we propose to examine these 14 variants in survivors with genome-wide data from the St. Jude Lifetime Cohort (SJLIFE) study [both original and expansion cohorts], CCSS Original Cohort and CCSS Expansion Cohort, separately. Association results from individual cohort will then be combined using a fixed-effect inverse-variance weighted meta-analysis. The concept proposal to examine in the SJLIFE study has already been approved and hence this request is to evaluate these 14 variants among the CCSS survivors from both Original and Expansion cohorts.

Specific Aims:

Aim 1: Evaluate the 14 PAVs (one at a time and in combination using the genetic risk score [GRS]) in each of the below cohorts, among survivors of European and African ancestries separately for their association with BMI

Aim 2: Examine the 14 PAVs in the four individual cohorts, with respect to exposure to CRT, glucocorticoid, sex and childhood obesity, when appropriate based on the numbers.

Aim 3: Meta-analyze the association results (in overall and stratified analyses) from individual cohorts, using a fixed-effect inverse-variance weighted method.

Aim 4: As a secondary analysis, assess the 14 PAVs for their potential associations with cardiometabolic traits, in individual cohorts and then meta-analyze the results (if possible).

Primary outcome of interest:

- First BMI measurement after the age of 18 years
- Genotype data of the 14 PAVs (directly genotyped or imputed [best-guess])

Covariates:

Age at cancer diagnosis
Sex
Age at BMI measurement
CRT dose
Glucocorticoid dose (dose if available, otherwise yes/no)
Cumulative anthracycline dose
Chest RT (maximum dose)
Pelvis RT (maximum dose)
Alkylating agents (classical) dose
Heavy metal alkylating agents dose
Childhood obesity (yes/no), if available
Race (to be used if principal components are not available)
Top 10 principal components (if available)

Secondary outcomes:

Hypertension
Stroke
Cardiomyopathy
Coronary Artery Disease
Type 2 diabetes
Cholesterol levels (HDL, LDL, triglycerides and total)
Systolic blood pressure
Diastolic blood pressure
Waist-to-hip ratio
Fasting Insulin levels

Analytic approach:

Associations between the 14 PAVs and BMI in each of the four study cohorts will be assessed using linear regression, adjusting for sex, age at diagnosis, age at BMI measurement, CRT dose,

Glucocorticoid dose, chest RT dose, pelvic RT dose, classical and heavy metal alkylating agents dose, childhood obesity, and ancestry-specific top 10 principal components. Analyses will be carried out separately among survivors of European and African ancestry as well as stratified by treatment exposures, sex and childhood obesity, wherever appropriate. Considering rarity of the 14 PAVs, statistical significance will be calculated based on 10000 random permutations of the genotype, and results with $P<0.05$ will be considered statistically significant.

We will perform a fixed-effect inverse-variance weighted meta-analysis of association results from four cohorts including survivors of European and African-descent separately first and then combine the results from both ancestries, if feasible. For variants with significant heterogeneity ($P<0.05$), meta-analysis will be carried out using random-effects model.

We will also create GRS in each cohort using weights of variants published in Turcot et al. *Nature Genetics* (2018) and examine the association between the GRS and BMI. GRS results will be meta-analyzed across the four cohorts using a fixed-effect model.

If feasible, we will assess the 14 PAVs for their associations with cardiovascular/cardiometabolic traits in each cohort separately and then meta-analyze the association results.

References

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