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Genomic determinants of long-term cardiometabolic complications in childhood acute lymphoblastic leukemia survivors

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Abstract

Background: While cure rates for childhood acute lymphoblastic leukemia (cALL) now exceed 80%, over 60% of survivors will face treatment-related long-term sequelae, including cardiometabolic complications such as obesity, insulin resistance, dyslipidemia and hypertension. Although genetic susceptibility contributes to the development of these problems, there are very few studies that have so far addressed this issue in a cALL survivorship context.

Methods: In this study, we aimed at evaluating the associations between common and rare genetic variants and long-term cardiometabolic complications in survivors of cALL. We examined the cardiometabolic profile and performed whole-exome sequencing in 209 cALL survivors from the PETALE cohort. Variants associated with cardiometabolic outcomes were identified using PLINK (common) or SKAT (common and rare) and a logistic regression was used to evaluate their impact in multivariate models.

Results: Our results showed that rare and common variants in the *BAD* and *FCRL3* genes were associated ($p < 0.05$) with an extreme cardiometabolic phenotype (3 or more cardiometabolic risk factors). Common variants in *OGFOD3* and *APOB* as well as rare and common *BAD* variants were significantly ($p < 0.05$) associated with dyslipidemia. Common *BAD* and *SERPINA6* variants were associated ($p < 0.05$) with obesity and insulin resistance, respectively.

Conclusions: In summary, we identified genetic susceptibility loci as contributing factors to the development of late treatment-related cardiometabolic complications in cALL survivors. These biomarkers could be used as early detection strategies to identify susceptible individuals and implement appropriate measures and follow-up to prevent the development of risk factors in this high-risk population.

Keywords: Acute lymphoblastic leukemia, cancer survivors, genetic determinants, cardiometabolic complications, genetic association study, extreme phenotype, obesity, dyslipidemia, insulin resistance, hypertension

Background

Childhood acute lymphoblastic leukemia (cALL) represents one third of all pediatric cancers [1]. Better understanding of the disease and treatment optimization over the last few decades has led to remarkable cure rates reaching 85% [2]. However, this therapeutic success comes at a substantial price since 60% of survivors currently face treatment-related long-term complications [3]. Children with cALL are exposed to chemo- and radiotherapy during a critical period of their development and thus have a greater risk of developing obesity [4], insulin resistance [2, 5], hypertension (HTN) [2, 6] and dyslipidemia [2], forming a metabolic syndrome (MetS) cluster [2]. These late treatment effects are worrisome since people affected by the MetS are at higher risk of atherosclerotic vascular disease [7], type 2 diabetes [8], and stroke [7]. The causes of these complications in cALL survivors remain unknown, but exposition to corticoids, methotrexate and cranial radiotherapy has been reported as contributing factor [9–12].

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In the general population, accumulating evidence indicate that nutrition has an important influence on MetS susceptibility and treatment response [13–17]. Furthermore, several susceptibility loci and genes are linked to MetS occurrence [13]. For instance, 20-40% of the variance of arterial blood pressure, insulin resistance, body mass index (BMI) and lipid levels are explained by genetic components [13, 18–22]. Genome-wide association studies (GWAS) revealed that genes coding for adipokines or proteins implicated in lipoprotein metabolism and inflammation are linked to the pathogenesis of MetS [13]. Obesity is influenced by variants in genes regulating food intake, energy metabolism and neuroendocrine pathways [18, 23, 24]. Numerous genes regulating β-cells function and insulin secretion explain a significant fraction of insulin resistance [25, 26], while variants in genes related to lipoprotein metabolism could explain up to 70% of lipid level inheritance [22, 27–29].

Despite their importance, only a few studies evaluating the cardiometabolic risk of cALL survivors have taken genetic factors into consideration [30–32]. The identification of genetic biomarkers could help pinpoint high-risk individuals and develop prevention strategies to counter the development of late cardiometabolic complications. Even with the success of GWAS in identifying genetic predisposition, only 10% of the genetic variance of complex diseases can be explained by common variants [26, 33]. The missing genetic contribution might be attributed to rare variants that were not captured by traditional GWAS [34, 35] or to the combined impact of rare and common variants [36]. With next-generation sequencing technologies, it is now possible to have simultaneously access to both common and rare variants for genetic association studies [37]. The aim of this study was to assess the contribution of both rare and common genetic variants in the prevalence of cardiometabolic complication in a cohort of cALL survivors.

Methods
Cohort
Participants included were treated for cALL at Sainte-Justine University Health Center (SJUHC, Montreal, Canada) with the Dana Farber Cancer Institute (DFCI) protocols [38]. The cALL survivors were recruited as part of the PETALE study at SJUHC and had an average of 15.5 years (+/- 5.2 SD) after diagnosis [39]. Subjects who were less than 19 years old at diagnosis, more than 5 years post diagnosis, free of relapse, and who did not receive hematopoietic stem cell transplantation were invited to participate. To limit heterogeneity, the emphasis was put on pre-B ALL since this type is the most frequent [40, 41]. Participants were mainly of French Canadian origin [42, 43]. During their medical visits, participants were subjected to a series of genetic and biochemical analyses and examined by a multidisciplinary team of health professionals including physicians, nutritionists, physiotherapists and psychotherapists. The study was approved by the Institutional Review Board of SJUHC and investigations were carried out in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from study participants or parents/guardians.

Classification of cardiometabolic risk factors
The presence of the cardiometabolic risk factors, obesity, insulin resistance, dyslipidemia and pre-HTN was assessed in all subjects. In adults, obesity was defined as a BMI ≥30 kg/m^2 and/or having a waist circumference ≥88 cm (women) or 102 cm (men) [44]. In children, BMI ≥97th percentile according to the BMI charts of the World Health Organization [45] and/or waist circumference ≥95th percentile defined obesity [46]. Blood pressure was measured on the right arm in the morning at rest. In adults, blood pressure ≥130/85 and <140/90 mmHg determined arterial pre-HTN and ≥140/90 mmHg HTN [47]. For children, we used current recommendations according to age and height: blood pressure ≥90th and <95th percentile indicated pre-HTN and ≥95th percentile HTN [48, 49]. Elevated fasting glucose, glycated hemoglobin (HbA1c) and/or homeostasis model assessment (HOMA-IR) were used to identify insulin resistance. Cut-off values were fasting glucose ≥6.1 mmol/L [50] and HbA1c ≥6% [50] for both adults and children. HOMA-IR ≥2.86 (adults) [2, 51] and ≥95th percentile for a pediatric reference population [52] were considered elevated. Dyslipidemia was defined based on high low-density lipoprotein-cholesterol (LDL-C), triglycerides (TG) and/or low high-density lipoprotein-cholesterol (HDL-C) concentrations. For adults, thresholds were LDL-C ≥3.4 mmol/L [53–55], TG ≥1.7mmol/L [53, 55, 56] and HDL-C <1.03 mmol/L in men and <1.3 in women [56]. For children, the values were compared to the National Heart, Lung and Blood Institute guidelines for age and gender [57]. Accumulation of cardiometabolic risk factors was determined by adding the presence of dyslipidemia, pre-HTN/HTN, insulin resistance and obesity. Participants with 3 or more risk factors were defined as "extreme phenotype" while those without risk factor were defined as "healthy".

Nutritional evaluation
Participants' dietary intakes were collected using a validated interviewer-administered food frequency questionnaire (FFQ) [58] combined with a 3-day food record. Evaluation of nutrient intakes was performed using the Nutrition Data System for Research software v.4.03 [59]. A validated Mediterranean score calculated

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Table 1 Estimated energy requirement equations

Group	Equation EER (kcal/d)
Boys 3-8 y	88.5 - (61.9 × age [y]) + PA × {(26.7 × weight [kg] + 903 × height [m])} + 20
Boys 9-18 y	88.5 - (61.9 × age [y]) + PA × {(26.7 × weight [kg] + 903 × height [m])} + 25
Men ≥19 y	662 - (9.53 × age [y]) + PA × {(15.91 × weight [kg]) + 539.6 × height [m])}
Girls 3-8 y	135.3 - (30.8 × age [y]) + PA × {(10.0 × weight [kg]) + 934 × height [m])} + 20
Girls 9-18 y	135.3 - (30.8 × age [y]) + PA × {(10.0 × weight [kg]) + 934 × height [m])} + 25
Women ≥19 y	354 - (6.91 × age [y]) + PA × {(9.36 × weight [kg]) + (726 × height [m])}

PA Physical activity coefficient, *y* years, *EER* estimated energy requirement

on a nine-point scale [60] was used to assess overall diet quality. Differences between calorie intake (calculated with the Institute of Medicine equations [61]) and estimated energy requirement (accounting for level of physical activity, equations shown in Table 1 [62]) determined energy balance.

Chemotherapeutic medication dose estimation
Theoretical cumulative doses of glucocorticoids (in prednisone equivalent $[mg/m^2]$), methotrexate (mg/m^2) and asparaginase (mg/m^2) were calculated for each participant according to DFCI treatment protocols [38].

Exposure and doses of cranial radiotherapy were recorded according to protocol.

Genetic data treatment and selection of variants
We performed whole-exome sequencing (WES) on a total of 209 participants from the PETALE cohort. Sequencing data were obtained from SJUHC and Génome Québec Integrated Centre for Pediatric Clinical Genomic using the SOLiD (ThermoFisher Scientific) or Illumina HiSeq 2500 platforms and were aligned on the Hg19 reference genome (Fig. 1). Rare and common variants with a predicted functional impact on protein were identified by the functional annotation from ANNOVAR [63]. Only variants with a PolyPhen-2 score ≥0.85 [64] or a SIFT score ≤0.1 [65, 66] were labeled as "potentially damaging" and used for further analyses. Two lists were assembled; the first was composed of genes involved in methotrexate and corticoid metabolic pathways [67] and few genes of lipid metabolism shown to affect corticosteroid-related complications such as hypertension or osteonecrosis [68, 69]. The second list contained genes related to cardiometabolic pathways that were selected based on gene ontology terms using GOrilla [70, 71] and DisGeNET [72–75]. Variants were defined as rare (minor allele frequency (MAF) <5%) and common (MAF ≥5%) according to the reported frequency in the 1000genome [76] and ESP6500 [77] datasets for Caucasian populations. A total of 198 variants in the cardiometabolic list and 7
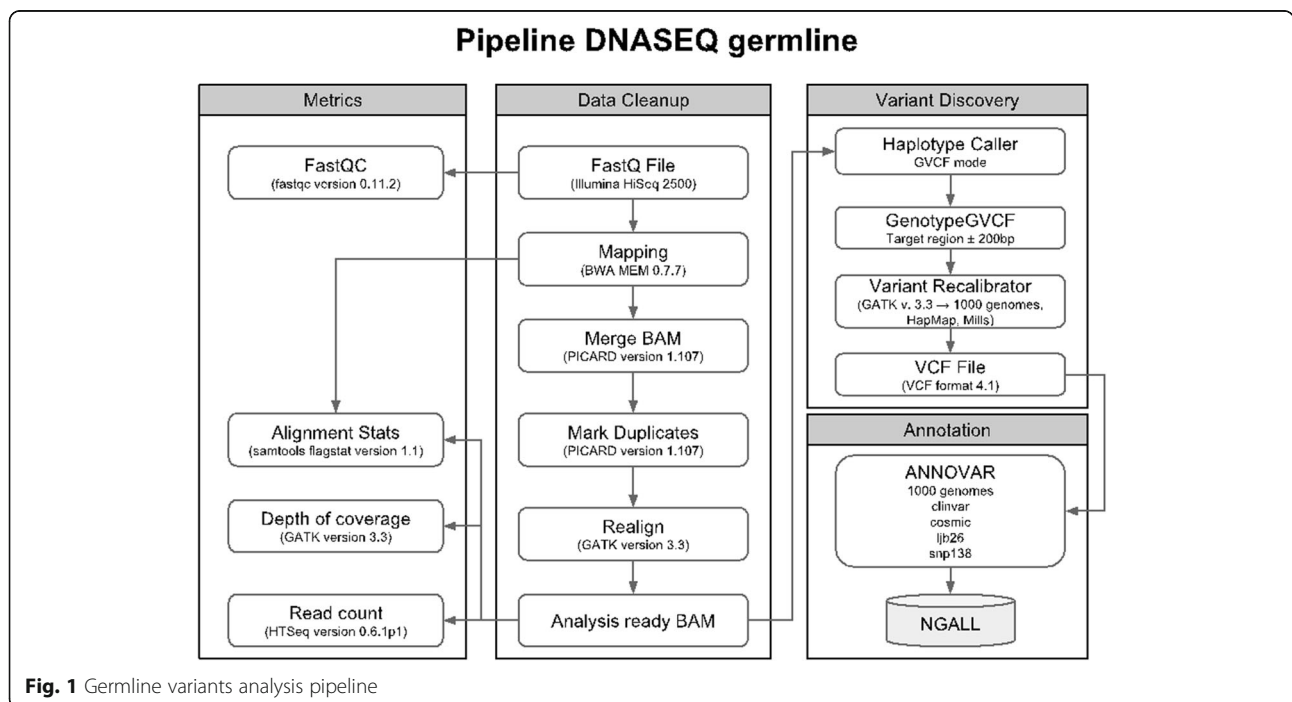

Fig. 1 Germline variants analysis pipeline

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variants in the methotrexate and corticoid list did not conform to the Hardy-Weinberg equilibrium and were rejected.

Power analysis

We used Quanto version 1.2.4 to compute power analysis at 80% [78] and Bonferroni correction for the number of SNPs or genes tested. The power analysis for common variant revealed that odds ratio (OR) ranging from 3 to 11 (depending on phenotype analyzed) for variants with MAF of 5-30% can be detected, whereas the lowest OR for rare variants, assuming a MAF of 0.01 that can be detected with a given sample size, was 16.

Association studies and statistical analyses

Association between cardiometabolic risk factors and common variants were studied using PLINK (http://zzz.bwh.harvard.edu/plink/) [79, 80]. For each association, we also determined the genetic model in which the common variant affects the phenotype: dominant model (one variant allele impacts the phenotype), recessive model (two variant alleles are needed to modify the phenotype) and additive model (accumulation of variant alleles causes a gradation in the risk of developing the phenotype). Association analyses of rare variants were performed using the SKAT-O test in the SKAT package (https://cran.r-project.org/web/packages/SKAT/index.html) [35] developed for the open software R [81]. Combined rare and common variant analyses were also done with the SKAT package. The Benjamini and Hochberg method (FDR) was used to correct for multiple testing for each list and variants with a FDR less than 0.20 were kept for further analyses [81]. Selected polymorphisms were analyzed using a logistic regression model including eight covariables: age at interview, gender, cumulative doses of corticoids, methotrexate and asparaginase, exposure or not to cranial radiotherapy, Mediterranean diet score and energy balance. Finally, we used chi-square tests to compare the prevalence of cardiometabolic complications between children and adults. Statistical analyses were performed using SPSS version 22.0 [82].

Results

Cohort characteristics

The characteristics of the cohort are presented in Table 2. The cohort (53.6% female) was mostly composed of adolescents and young adults (median age of 22.4 years). Dyslipidemia was the most prevalent cardiometabolic risk factor (41.8%), followed by obesity (33.0%), insulin resistance (18.5%) and pre-HTN (10.1%). Dyslipidemia was the only risk factor for which we

Table 2 Characteristics of the PETALE cohort

	Total cohort	Adults	Children	*p*-value
Gender, n (%)				
Male	97 (46.4)	68 (46.6)	29 (46.0)	0.942
Female	112 (53.6)	78 (53.4)	34 (54.0)	
Age, median (range)	22.4 (8.5-41.0)	24.9 (18.1-41.0)	16.2 (8.5-17.9)	
Phenotype, n (%)				
Obesity	69 (33.0)	48 (32.9)	21 (33.3)	0.949
Pre-hypertension	21 (10.1)	16 (10.9)	5 (7.9)	0.505
Insulin resistance	38 (18.5)	29 (20.1)	9 (14.5)	0.34
Dyslipidemia	87 (41.8)	68 (46.9)	19 (30.2)	0.025
Extreme phenotype	22 (10.7)	18 (12.5)	4 (6.5)	0.197
Number of risk factors				
0	81 (39.3)	51 (35.4)	30 (48.4)	0.388
1	62 (30.1)	45 (31.3)	17 (27.4)	
2	41 (19.9)	30 (20.8)	11 (17.7)	
3	19 (9.2)	16 (11.1)	3 (4.9)	
4	3 (1.5)	2 (1.4)	1 (1.6)	

Extreme phenotype: Three and more cardiometabolic risk factor
Chi-square tests were used to compare the prevalence of cardiometabolic complications between children and adults

observed a significant difference between children and adults (30.2% vs. 46.9%, *P*<0.025). Of note, less than 40% of the cohort was classified as "healthy" (no MetS risk factor) and 10.7% as "extreme phenotype" (≥3 MetS risk factors).

Genetic associations with cardiometabolic candidate genes

We analyzed 1,202 common variants from the cardiometabolic candidate gene list (Fig. 2). We found associations between common variants and two phenotypes (Table 3): dyslipidemia and the extreme phenotype. Eukaryotic Translation Initiation Factor 4B (*EIF4B*) (FDR 0.18) and 2-oxoglutarate and iron dependent oxygenase domain containing 3 (*OGFOD3*) (FDR 0.18) was associated with dyslipidemia while extreme phenotype was linked to BCL2 Associated Agonist Of Cell Death (*BAD*) (FDR 0.20) and Fc Receptor Like 3 (*FCRL3*) (FDR 0.20). The SKAT-O test performed on the 12,977 rare variants did not reveal any significant association. The rare/common variant combined analysis showed associations between the extreme phenotype and 3 genes: *BAD* (FDR 0.09), *FCRL3* (FDR 0.09) and *EIF4B* (FDR 0.10) (Table 3).

Genetic associations with methotrexate and corticosteroid candidate genes

Next, we studied 34 common variants in the methotrexate/corticoid candidate gene list (Fig. 3). For dyslipidemia, we observed associations with *BAD* (FDR 0.02) and Apolipoprotein B (*APOB*) (FDR 0.11) (Table 4). *BAD* was also associated with the extreme phenotype (FDR 0.009), insulin resistance (FDR 0.07) and obesity

Fig. 2 Processing of single nucleotide polymorphism for cardiometabolic candidate genes
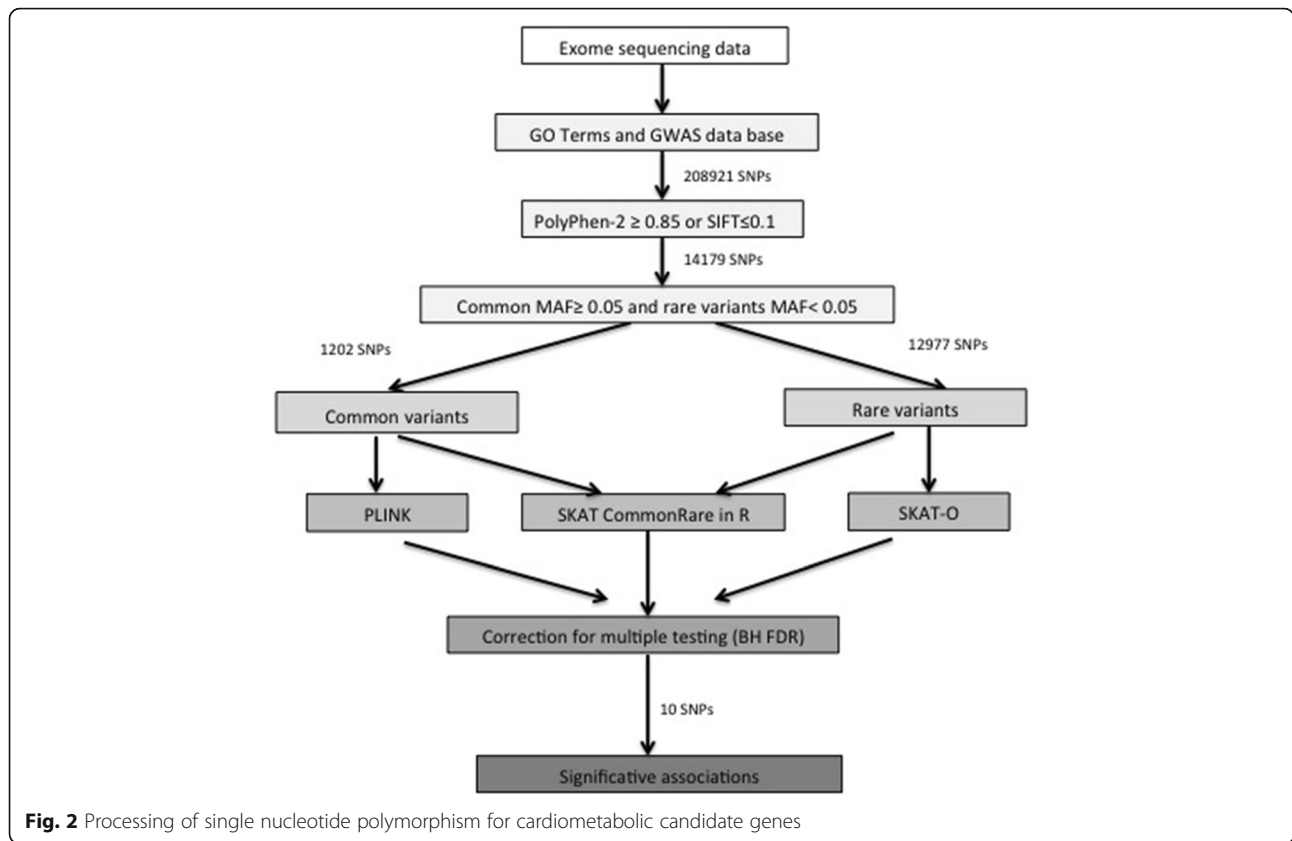
(FDR 0.08). Moreover, insulin resistance was associated with a common variant in Serpin Family A Member 6 (*SERPINA6*) (FDR 0.07) (Table 4). The SKAT-O analysis for 376 rare variants revealed associations between glucocorticoid receptor (Nuclear Receptor Subfamily 3 Group C Member 1, *NR3C1*, FDR 0.17) and the extreme phenotype as well as between pre-HTN and Corticotropin Releasing Hormone Receptor 1 (*CRHR1*) (FDR 0.20) and Corticotropin Releasing Hormone Receptor 2 (*CRHR2*) (FDR 0.20) (Table 4). Combined rare and common variant analyses exhibited 8 associations: *BAD* (FDR 0.04), *APOB* (FDR 0.12), Cystathionine-Beta-Synthase (*CBS*) (FDR 0.12) and Solute Carrier Organic Anion Transporter Family Member 4C1 (*SLCO4C1*) (FDR 0.14) with dyslipidemia; *BAD* (FDR 0.003) and *NR3C1* (FDR 0.15) with the extreme phenotype; and *CRHR1* (FDR 0.14) and *CRHR2* (FDR 0.14) with pre-HTN (Table 4).

Table 3 Significant genetic associations with cardiometabolic candidate genes

Common Variants							
	Gene	SNP ID		MAF	*p*-value	FDR	Model
Dyslipidemia	*EIF4B*	rs146008363		0.05	0.00018	0.180	DOM
	OGFOD3	rs62079523		0.33	0.00032	0.180	DOM
Extreme phenotype	*BAD*	rs2286615		0.10	0.00034	0.200	DOM
	FCRL3	rs2282284		0.03	0.00042	0.200	DOM
Common/Rare variants							
	Gene	Rare (n)	Common (n)	*p*-value	FDR		
Extreme phenotype	*BAD*	3	1	5.79×10^{-5}	0.087		
	FCRL3	2	1	3.86×10^{-5}	0.087		
	EIF4B	1	1	0.00010	0.100		

MAF Minor allele frequency, *DOM* Dominant effect, *Rare (n)* Number of rare variants analyzed in the gene, *Common (n)* Number of common variants analyzed in the gene, *Extreme phenotype* Three and more cardiometabolic risk factor
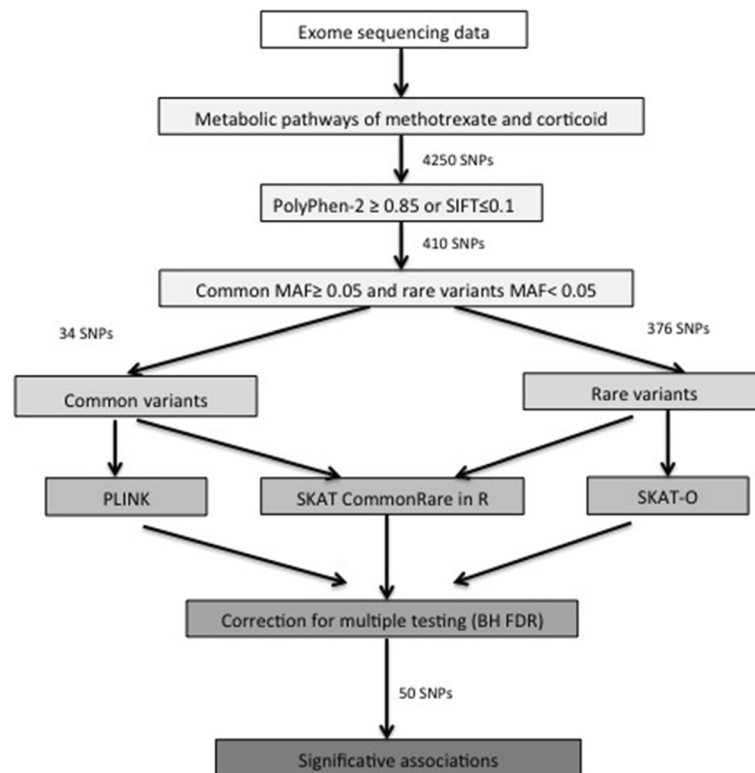
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Fig. 3 Processing of single nucleotide polymorphism for methotrexate and corticoid pathways' candidate genes

Logistic regression analysis with significant cardiometabolic candidate genes

Significant genetic variants were further analyzed in a logistic regression model including 8 covariables (see Methods). Analysis revealed independent associations between the extreme phenotype and the common variant rs2286615 in *BAD* (p=0.006, in a dominant effect model), age at interview (p=0.04), and exposure to cranial radiotherapy (p=0.04) (Table 5). The common and rare variant analysis showed associations between the extreme phenotype and age (p=0.03), cumulative doses of methotrexate (p=0.05), exposure to cranial radiotherapy (p=0.04) and the *BAD* gene (p=0.003) (Table 5). The common variant rs2282284 in *FCRL3* was also associated with the extreme phenotype with a dominant effect (p=0.006) (Table 5). *FCRL3* (rare and common variants) was associated with the extreme phenotype (p=0.04) while no other covariable reached statistical significance in this model (Table 5). The variant rs62079523 in *OGFOD3*, associated with dyslipidemia in the dominant model, was found highly significant in the logistic regression model (p=0.005) (Table 5).

Logistic regression model with significant methotrexate and corticoid candidate genes

The results of the logistic regression analyses for the significant genes in the methotrexate/corticosteroid list are presented in Table 6. We found that the common *BAD* variant rs2286615 was associated with the extreme phenotype (p=0.006) in a dominant and additive effect as it was with age (p=0.04) and cranial radiotherapy (p=0.04). The combined analysis of common and rare *BAD* variants was significant for the extreme phenotype (p=0.003). In this model, age (p=0.03), cumulative doses of methotrexate (p=0.05) and cranial radiotherapy (p=0.04) were also significant. *BAD* was associated with dyslipidemia for the common variant rs2286615 (p=0.008, additive model) and for the common and rare variants (p=0.006). Also the rs2286615 variant was associated in dominant (p=0.009) and additive (p=0.006) effect model with the presence of obesity. Rs676210, a variant in *APOB*, had a dominant effect on the risk of dyslipidemia and was the only significant association in the logistic regression model (p=0.02). An additive effect was observed for the common variant rs2228541 (*SERPINA6*) and insulin resistance (p=0.05). Finally, the logistic regression model including rare variants in *CRHR1* and *CRHR2* for pre-HTN revealed associations for gender (p=0.03) but the genetic associations did not reach statistical significance.

Discussion

This study is among the first studies to address the contribution of genetic determinants in the development of

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Table 4 Significant genetic associations with methotrexate and corticosteroid candidate genes

Common Variants

	Gene	SNP ID	MAF	*p*-value	FDR	Model
Dyslipidemia	BAD	rs2286615	0.10	0.00065	0.021	ADD
	APOB	rs676210	0.23	0.0069	0.110	DOM
Extreme phenotype	BAD	rs2286615	0.10	0.00034	0.0089	ADD, DOM
Insulin resistance	BAD	rs2286615	0.10	0.0044	0.069	DOM
	SERPINA6	rs2228541	0.50	0.0051	0.069	ADD, DOM, REC
Obesity	BAD	rs2286615	0.10	0.0025	0.081	ADD, DOM

Rare variants

	Gene	Rare (n)		*p*-value	FDR	
Extreme phenotype	NR3C1	2		0.0021	0.17	
Pre-hypertension	CRHR1	1		0.0025	0.20	
	CRHR2	2		0.0048	0.20	

Common/Rare variants

	Gene	Rare (n)	Common (n)	*p*-value	FDR	
Dyslipidemia	BAD	3	1	0.00049	0.040	
	APOB	30	3	0.0028	0.12	
	CBS	3	0	0.0042	0.12	
	SLCO4C1	4	0	0.0066	0.14	
Extreme phenotype	BAD	3	1	3.35×10^{-5}	0.0028	
	NR3C1	2	0	0.0037	0.15	
Pre-hypertension	CRHR1	1	0	0.0032	0.14	
	CRHR2	2	0	0.0033	0.14	

MAF Minor allele frequency, *DOM* Dominant effect, *ADD* Additive effect, *REC* Recessive effect, *Rare (n)* Number of rare variants analyzed in the gene, *Common (n)* Number of common variants analyzed in the gene, *Extreme phenotype* Three and more cardiometabolic risk factor

long-term cardiometabolic complications in cALL survivors. Globally, we found that the development of an extreme cardiometabolic phenotype can be predicted by common and rare variants in *BAD* and *FCRL3*. The presence of dyslipidemia in cALL survivors is influenced by common variants in *OGFOD3* and *APOB* and by common and rare variants in *BAD*. Obesity was predicted by a common variant in *BAD* and insulin resistance was associated with a common variant in *SERPINA6*. Pre-HTN was related to survivors' gender as being a female was found protective for this complication. This gender difference between men and women before menopause has been well described in the literature [83, 84].

We found similar prevalence of obesity in children and in adults, suggesting that obesity acquired during childhood following the treatments persists thorough adulthood, a hypothesis supported by other studies [85–87]. Obesity is central to the MetS and is a major risk factor for HTN, dyslipidemia and insulin resistance [23, 88]. The PETALE cohort appeared to be particularly affected by dyslipidemia as almost 47% of adults were afflicted. For comparison, a study conducted in a population of young Canadian adults (18-39 years old) revealed that 34% were affected by dyslipidemia [89]. Given their young age, this finding raises concerns for the long-term cardiovascular risk of cALL survivors. In fact, 60% of our cohort was affected by at least one cardiometabolic risk factor, 10.7% of them being classified as extreme phenotypes. The observation related to the median age of 22.4 years places the survivors at high risk for early cardiovascular disease.

The common variant rs2286615 in the *BAD* gene was associated with extreme phenotype and obesity, whereas interactions between rare and common variants were linked to extreme phenotype and dyslipidemia. *BAD* is a gene that codes for a protein member of the pro-apoptotic Bcl-2 protein family named "Bcl2-associated agonist of cell death". In response to activation by hypoxia, reactive oxygen species, nutrient withdrawal or DNA damage, the pro-apoptotic proteins in the Bcl-2 family create pores in the mitochondrial membrane by which cytochrome can be released, triggering the apoptotic cascade leading to cell death [90]. *BAD* could have an impact on the development of insulin resistance since an imbalance between pro-apoptotic and anti-apoptotic proteins in situation of high blood glucose promotes β-

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Table 5 Logistic regression model with significant cardiometabolic candidate genes

	Extreme Phenotype				Dyslipidemia
	BAD/rs2286615 (C, DOM)	FCRL3/rs2282284 (C,DOM)	BAD (CR)	FCRL3 (CR)	OGFOD3/rs62079523 (C, DOM)
	OR (95% CI) p-value				
Age	1.219 (1.005-1.478)	1.151 (0.993-1.334)	1.213 (1.017-1.447)	1.150 (0.993-1.332)	1.033 (0.962-1.109)
	0.044	0.062	**0.032**	0.062	0.374
Gender	1.152 (0.216-6.142)	1.062 (0.268-4.201)	1.624 (0.340-7.749)	1.039 (0.266-4.063)	0.720 (0.360-1.439)
	0.869	0.932	0.543	0.956	0.352
Corticoid	1.000 (1.000-1.000)	1.000 (1.000-1.000)	1.000 (1.000-1.000)	1.000 (1.000-1.000)	1.000 (1.000-1.000)
	0.577	0.570	0.355	0.574	0.528
Asparaginase	1.000 (1.000-1.000)	1.000 (1.000-1.000)	1.000 (1.000-1.000)	1.000 (1.000-1.000)	1.000 (1.000-1.000)
	0.714	0.158	0.444	0.270	0.346
Methotrexate	0.999 (0.999-1.000)	1.000 (0.999-1.000)	0.999 (0.999-1.000)	1.000 (0.999-1.000)	1.000 (1.000-1.000)
	0.075	0.800	**0.048**	0.729	0.696
CRT	14.506 (1.116-188.530)	4.938 (0.687-35.491)	16.098 (1.220-212.463)	3.544 (0.561-22.385)	1.708 (0.668-4.366)
	0.041	0.112	**0.035**	0.178	0.264
Energy balance	0.999 (0.998-1.001)	0.999 (0.998-1.000)	1.000 (0.998-1.001)	0.999 (0.999-1.000)	1.000 (0.999-1.000)
	0.297	0.304	0.421	0.306	0.210
Med score	0.652 (0.319-1.329)	0.884 (0.518-1.509)	0.752 (0.374-1.513)	0.815 (0.491-1.353)	1.008 (0.807-1.259)
	0.239	0.651	0.425	0.430	0.944
SNP	57.900 (3.152-1063.462)	67.983 (3.393-1362.288)	68.819 (4.202-1159.995)	11.695 (1.150-118.907)	2.712 (1.352-5.442)
	0.006	**0.006**	**0.003**	**0.038**	**0.005**

Top: Odds ratio [95% CI], bottom: *p*-value
Boldface: significant association
C common, *CR* common/rare, *DOM* Dominant effect, *CRT* Cranial radiotherapy, *Med score* Mediterranean diet score, *Extreme phenotype* Three and more cardiometabolic risk factor

cell apoptosis [90], the latest playing an important role in the pathophysiology of type 2 diabetes [90]. Studies suggest that *BAD* has a role in β-cell function and can promote glucose-stimulated insulin secretion [91–93]. Besides, it has been reported that *BAD* suppresses the formation of tumors in lymphocytes and that *Bad*-deficient mice are at higher risk of lymphoma and leukemia [94]. In another study, *Bad*-deficient mice were prone to cancer and did not respond adequately to DNA damage [95]. This gene is thus a suitable candidate to explain a common etiology between the predisposition to cardiometabolic complication and hematologic malignancies. Because *BAD* is recurrent in almost all associations with the cardiometabolic risk factors in our study, we can conclude that it is a strong candidate gene for MetS in cALL survivors. It is possible that through its effects on insulin resistance, *BAD* can predispose the participants to develop obesity, dyslipidemia and pre-HTN [8, 96–98]. As expected, age had an impact on the presence of the extreme phenotype in the model with *BAD*. We observed that adults were more affected by cardiometabolic complications than children. This can be explained by the fact that the establishment of cardiometabolic risk factors is a long-term and latent process. Other studies on cALL survivors have reported that obesity, diabetes and the metabolic syndrome are more frequent in patients who received cranial radiotherapy [9, 10, 99]. This is in accordance with our results showing that cranial radiotherapy significantly increased the risk of extreme phenotype. This could be caused by the impact of radiotherapy on the brain satiety control center and on hormones implicated in energy regulation [1, 100, 101]. Indeed, damages caused by cranial radiotherapy could lead to growth hormone deficiency and then to the development of metabolic disorders such as visceral obesity, hyperinsulinemia and low HDL-C [102].

Carriers of one allele of the variant rs2282284 in *FCRL3*, encoding for a protein that is part of the immunoglobulin receptors, were at increased risk of presenting the extreme phenotype. The common and rare variant analysis also revealed a significant association between *FCRL3* and the extreme phenotype. It has a role in immune function and is expressed in secondary lymphoid organs, mostly in B lymphocytes [103]. This gene has been linked to rheumatoid arthritis, autoimmune thyroid disease and systemic lupus erythematosus [103–105]. In particular, the SNP rs2282284 has been associated to higher risk of

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Table 6 Logistic regression model with significant methotrexate and corticoid candidate genes

Values shown as OR (95% CI) / p-value.

	Extreme phenotype		Dyslipidemia		
	BAD/rs2286615 (C, DOM, ADD)	BAD (CR)	APOB/rs676210 (C, DOM)	BAD/rs2286615 (C, ADD)	BAD (CR)
Age	1.219 (1.005-1.478) / **0.044**	1.213 (1.017-1.447) / **0.032**	1.041 (0.971-1.117) / 0.259	1.047 (0.961-1.141) / 0.292	1.037 (0.966-1.114) / 0.313
Gender	1.152 (0.216-6.142) / 0.869	1.624 (0.340-7.749) / 0.543	0.726 (0.365-1.444) / 0.361	1.058 (0.453-2.472) / 0.896	1.043 (0.506-2.151) / 0.908
Corticoid	1.000 (1.000-1.000) / 0.577	1.000 (1.000-1.000) / 0.355	1.000 (1.000-1.000) / 0.411	1.000 (1.000-1.000) / 0.519	1.000 (1.000-1.000) / 0.571
Asparaginase	1.000 (1.000-1.000) / 0.714	1.000 (1.000-1.000) / 0.444	1.000 (1.000-1.000) / 0.372	1.000 (1.000-1.000) / 0.704	1.000 (1.000-1.000) / 0.344
Methotrexate	0.999 (0.999-1.000) / 0.075	0.999 (0.999-1.000) / **0.048**	1.000 (1.000-1.000) / 0.783	1.000 (1.000-1.000) / 0.225	1.000 (1.000-1.000) / 0.642
CRT	14.506 (1.116-188.530) / 0.041	16.098 (1.220-212.463) / 0.035	1.572 (0.619-3.994) / 0.341	2.361 (0.751-7.425) / 0.142	2.255 (0.843-6.033) / 0.105
Energy balance	0.999 (0.998-1.001) / 0.297	1.000 (0.998-1.000) / 0.421	1.000 (0.999-1.000) / 0.469	1.000 (0.999-1.000) / 0.319	1.000 (0.999-1.000) / 0.350
Med score	0.652 (0.319-1.329) / 0.239	0.752 (0.374-1.513) / 0.425	1.017 (0.813-1.272) / 0.885	1.029 (0.793-1.334) / 0.831	0.984 (0.780-1.240) / 0.888
SNP	57.900 (3.152-1063.462) / **0.006**	69.819 (4.202-1159.995) / **0.003**	0.434 (0.215-0.877) / **0.020**	4.022 (1.441-11.226) / **0.008**	3.560 (1.427-8.882) / **0.006**

	Obesity	Pre-hypertension		Insulin resistance
	BAD/rs2286615 (C, DOM)	CRHR1 (R)	CRHR2 (R)	SERPINA6/rs2228541 (C, ADD)
Age	0.996 (0.914-1.085) / 0.926	1.010 (0.873-1.169) / 0.896	0.995 (0.869-1.139) / 0.940	1.085 (0.993-1.185) / 0.073
Gender	1.979 (0.824-4.750) / 0.127	0.081 (0.009-0.741) / **0.026**	0.165 (0.033-0.809) / **0.026**	1.330 (0.562-3.150) / 0.517
Corticoid	1.000 (1.000-1.000) / 0.998	1.000 (1.000-1.000) / 0.828	1.000 (1.000-1.000) / 0.830	1.000 (1.000-1.000) / 0.898
Asparaginase	1.000 (1.000-1.000) / 0.863	1.000 (1.000-1.000) / 0.621	1.000 (1.000-1.000) / 0.452	1.000 (1.000-1.000) / 0.141

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Table 6 Logistic regression model with significant methotrexate and corticoid candidate genes *(Continued)*

	Extreme phenotype	Dyslipidemia	
Methotrexate	1.000 (1.000-1.000)	1.000 (0.999-1.000)	1.000 (1.000-1.000)
	0.825	0.364	0.935
CRT	1.915 (0.607-6.038)	3.558 (0.550-23.009)	1.539 (0.463-5.118)
	0.267	0.183	0.482
Energy balance	1.000 (0.999-1.000)	0.999 (0.998-1.000)	1.000 (0.999-1.000)
	0.137	0.168	0.257
Med score	0.921 (0.710-1.195)	0.806 (0.555-1.170)	0.939 (0.713-1.236)
	0.534	0.257	0.652
SNP	3.993 (1.410-11.307)	3.417 (0.384-30.405)	0.534 (0.286-0.998)
	0.009	0.271	**0.049**

Hidden column (Dyslipidemia second):
| 1.000 (0.999-1.000) |
| 0.160 |
| 7.685 (0.713-82.885) |
| 0.093 |
| 1.000 (0.999-1.001) |
| 0.573 |
| 0.871 (0.562-1.349) |
| 0.536 |
| 76.406 (0.948-6158.616) |
| 0.053 |

Top: Odds ratio (95% CI), bottom: *p*-value
Boldface: Significant association
C common, *CR* common/rare, *R* rare, *DOM* Dominant effect, *ADD* Additive effect, *CRT* Cranial radiotherapy, *Med score* Mediterranean diet score, *Extreme phenotype* Three and more cardiometabolic risk factor

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neuromyelitis optica (a severe inflammatory demyelinating disease of the central nervous system) [106] and correlated with the risk of multiple sclerosis [107] in the Chinese Han population. *FCRL3* role in immune regulation is of interest given the contribution of inflammation in MetS pathogenesis [7, 108, 109].

The common variant rs62079523 in *OGFOD3* was found associated with dyslipidemia in the dominant model. No clear function has been reported for this gene in the literature but it was linked with the gene ontology term 2-oxoglutarate and iron-dependent oxygenase domain-containing protein 3 in our analysis.

We found the common variant rs676210 in *APOB* correlated with the development of dyslipidemia, the presence of the minor allele (A) being protective for the outcome. *APOB* codes for the apolipoproteins B-48 and B-100 that play a central role in lipid transport and metabolism. They are the main apolipoproteins of chylomicron, very low density lipoprotein (VLDL) and LDL [110, 111]. The rs676210 polymorphism induces a change (proline to leucine) in position 2739 of the protein, thereby not affecting apolipoprotein B-48, a 2152 amino acid protein that is the result of *APOB* RNA editing [112, 113]. In line with our results, it was demonstrated that the carriers of the major allele (G) had higher levels of oxidized LDL [114, 115] that predispose to atherosclerosis. However, these studies failed to find an association between the SNP and risk of cardiovascular events [114]. Moreover, in comparison with the carriers of the major allele G, the minor allele A was linked to lower TG, total cholesterol and LDL-C levels and with higher HDL-C [114]. This profile is favorable to a healthy cardiovascular system [114] and is in agreement with our findings. A study also reported a higher prevalence of glucocorticoid-induced hypertension in patients with an *APOB* polymorphism [68], which demonstrate the multiple impacts this gene can have on cardiovascular health.

The variant rs2228541 in *SERPINA6* was associated with a decreased risk of insulin resistance. Similarly, common variants at the *SERPINA6* locus were found associated with plasma levels of cortisol in a study comprising of 12,597 Caucasians [116]. It was postulated that this effect was mediated by changes in the total cortisol binding capacity by the corticosteroid binding globulin. Variations in plasma cortisol levels have been associated with cardiovascular disease, obesity, type 2 diabetes, HTN and dyslipidemia [116]. Thus, this SNP could be linked to cortisol levels and thus predisposes to type 2 diabetes. However, because data was not available, we could not determine if *SERPINA6* variants were associated with the development of hyperglycemia during ALL treatment.

Rare variants in the *CRHR1* and *CRHR2* genes were linked to pre-HTN. This effect was lost in the logistic regression model, but the latter uncovered the impact of gender on the phenotype, women being protective for the outcome. The unequal distribution of the phenotype between the genders (17.53% in men and 3.57% in women) could probably explain the observed relationship.

On the other hand, corticoid and asparaginase cumulative doses did not have a significant impact on the development of cardiometabolic risk factors in our study. It appeared that exposure to cranial radiotherapy was the major risk factor to predict the development of late cardiometabolic complications. Moreover, neither the quality of diet (evaluated with the Mediterranean diet score) nor the excess in calories were found significantly associated with the outcomes in our models.

Standard contingency tables and regression model allowed us to study common variants but did not provide enough power to study rare variants [36]. We had to use a technique that analyzes the cumulative effects of different rare variants on the same gene [117]. We also performed combined rare and common variants analysis in order to detect interactions. With this strategy we were able to discover associations that could not be seen with traditional associations studies, consisting the strength of this study. The limited sample size did not provide us with optimal power, especially for rare variants analysis. Replication studies in other cohorts of cALL survivors will be needed to confirm the observed associations.

Conclusions

This study contributes to better understand the genetic determinants in the development of long-term cardiometabolic complication in childhood ALL survivors. Genetic information associated with both common and rare variants can help predict the development of late onset cardiometabolic complications. Genetic biomarkers can be used to propose prevention strategies, personalize the treatment and the follow-up to minimize the long-term sequelae and increase the quality of life of this high-risk population.

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Availability of data and materials

The datasets are available from the corresponding author upon request.

Authors' contributions

DS, MK, EL, SD, VM and CL conceived the study and participated in the design and coordination. VM collected the cardiometabolic data, VM and JE classified participants according to their metabolic status. PSO and PB processed the genetic data of the PETALE survivors. JE did the genetic association studies and the logistic regression model and interpreted the data. JE, VM, SD, EL and DS contributed to the writing of the manuscript. All authors have read and approved this manuscript.

Ethics approval and consent to participate

The study was approved by the Institutional Ethics Review Board of Sainte-Justine UHC. Written informed consent was obtained from study participants and/or parents/guardians.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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