Research Article

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Pharmacogenomics

Genes identified through genome-wide association studies of osteonecrosis in childhood acute lymphoblastic leukemia patients

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Aim: To evaluate top-ranking genes identified through genome-wide association studies for an association with corticosteroid-related osteonecrosis in children with acute lymphoblastic leukemia (ALL) who received Dana–Farber Cancer Institute treatment protocols. **Patients & methods:** Lead SNPs from these studies, as well as other variants in the same genes, pooled from whole exome sequencing data, were analyzed for an association with osteonecrosis in childhood ALL patients from Quebec cohort. Top-ranking variants were verified in the replication patient group. **Results:** The analyses of variants in the *ACP1-SH3YL1* locus derived from whole exome sequencing data showed an association of several correlated SNPs (rs11553746, rs2290911, rs7595075, rs2306060 and rs79716074). The rs79716074 defines *B haplotype of the *APC1* gene, which is well known for its functional role. **Conclusion:** This study confirms implication of the *ACP1* gene in the treatment-related osteonecrosis in childhood ALL and identifies novel, potentially causal variant of this complication.

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Osteonecrosis (ON) is one of the major complications of childhood acute lymphoblastic leukemia (ALL) treatment [1–3]. It can be symptomatic and cause severe pain, joint damage and articular collapse or can remain asymptomatic and cause no disabilities. Although corticosteroids (CS) like prednisone (PDN) and dexamethasone (DXM) are widely used in ALL protocols [1,4–6] for their capacity to induce apoptosis of leukemia cells, they can also lead to symptomatic ON in a subset of patients [7–11]. It is a dose-limiting toxicity that can precipitate early withdrawal of CS from therapy for ALL [12]. Other factors may potentiate the effect of CS like concomitant drugs used in ALL therapy [13] or clinical prognostic factors, such as age at diagnosis higher than 10 years [1], female gender [2] and higher body mass index [14]. In order to detect the genetic component underlying the risk of ON, which may lead to personalized treatment or prophylactic options, several genome-wide association studies (GWAS) have been conducted recently, linking a number of polymorphisms to ON development [12,15,16]. For example, variants were identified near the glutamate receptor *GRIN3A* locus [12], near *BMP7* [15] and in *ACP1* gene [16].

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Given that replication of GWAS hits is essential to evaluate their applicability to different treatment protocols or populations, here we evaluated whether the top-ranking GWAS hits reported in these studies are also predictors of ON development in ALL patients of European descent treated with Dana–Farber Cancer Institute (DFCI) protocols. In addition, other variants located in the same genes were pooled from whole exome sequencing (WES) data and analyzed for an association with ON.

Patients & methods

Study population

The discovery cohort, Quebec childhood acute lymploblastic leukemia (QcALL), consisted of 297 Caucasian children and adolescent from Quebec diagnosed with ALL (incident cases) at the University Health Center Sainte-Justine, Montreal, QC, Canada. Patients were treated in accordance with DFCI ALL Consortium protocols (DFCI 87-01, 91-01, 95-01 or 00-01) between January 1987 and July 2005 [17]. The replication cohort (DFCI) consisted of 177 nonincident Caucasian patients who underwent treatment with DFCI 00-01 protocol in the nine remaining DFCI Consortium Institutions. Patients with available clinical data and DNA samples that allowed successful genotyping were included in the study (Supplementary Figure 1). Description of these cohorts and DFCI treatment regimens is provided elsewhere [4–6,17–19]; patients' characteristics are also given in Supplementary Table 1. Briefly, all patients received PDN during the induction phase (40 mg/m^2/day); CS were administered during the intensification and continuation phases as 5-day pulses every 3 weeks until the completion of therapy. On protocols 87-01 and 95-01, PDN was used during these treatment phases, on Protocol 91-01, DXM was used instead of prednisone and on Protocol 00-01, patients were randomized to receive either PDN or DXM. Standard-risk (SR) patients received DXM at a dose of 6 mg/m^2/day or PDN at a dose of 40 mg/m^2/day and high-risk (HR) patients received doses three-times higher than those received by SR patients during both the intensification and continuation phases, except on protocol 00-01 when HR patients received the same dose as SR patients during the continuation phase [18]. Information on symptomatic ON (corresponding to NCI grades 2–4) [20] in QcALL was collected from the patients' medical files and was defined as persistent bone pain or motor function limitations associated with ON lesion confirmed by radiological examination such as x-ray radiography, computed tomography scan or magnetic resonance imaging. For the DFCI patients, ON was reported by each participating institution as part of bony morbidity.

Genotyping

GWAS hits [12,15,16] have been investigated for an association with symptomatic ON in QcALL cohort; 16 SNPs were reported as top-ranking SNPs associated with ON (Supplementary Tables 2 & 3) [12,15,16]. *ACP1/SHY3L1* locus was the only top-ranking locus reported by Kawedia *et al.* [16]; several SNPs associated with ON were identified in that study with p ranging from 1.2×10^{-6} to 4.6×10^{-6}. Remaining SNPs were lead SNPs reported in GWAS by Karol *et al.* in 2015 and 2016 [12,15]. From Karol *et al.* [12,15], we included all SNPs reported by the authors as top-ranking SNPs from meta-analysis of several cohorts (provided in the main table of the article, p range from 2.7×10^{-8} to 8.6×10^{-6}). Similar reasoning, while keeping the same p-value range, was followed for the selection of SNPs from Karol *et al.* [12,15]. Three SNPs with MAF lower than 5% in the population of European ancestry were excluded, resulting in 13 SNPs included in the analyses (Table 1). The genotypes were obtained at Sequenom platform at the McGill University and Genome Quebec Innovation Centre or by PCR-coupled allele specific oligonucleotide hybridization assay [18,21]. In addition, other variants located in the same genes, were pooled from WES data, which were available for 277 patients of QcALL cohort [22]. For the common SNP analyses, exonic variants that had MAF lower than 5%, with missing rate higher than 20% or not in Hardy–Weinberg equilibrium ($p < 0.001$) were excluded from the analysis, resulting in 43 SNPs located in 12 genes that were included in the association analyses with ON (Supplementary Table 4). Positively associated SNPs were subsequently genotyped in all patients of QcALL cohort and analyzed in the replication DFCI cohort. The rare variants (MAF < 5%) from WES data were also analyzed in the same set of genes using collapsing approach whereby minor alleles of at least two variants within one gene, each in Hardy–Weinberg equilibrium and with missing rate <20% are analyzed together (Supplementary Table 5).

Statistical analysis

Association of genotypes with the presence of ON (at least one incident during the 2 years of therapy) was assessed by χ^2 test or Fisher exact test in all patients and in patients assigned to the HR group, given the higher CS doses

Table 1. Results of the analyses of genome-wide association studies SNP genotyped in Quebec childhood acute lymploblastic leukemia cohort.

Gene	dbSNP_id	SUBST.	GEN.	Affected	Nonaffected	OR (95% CI)	p-value
ACP1†	rs12714403	G>A	GG	25 (80.6)	211 (80.5)	1	0.99
			GA, AA	6 (19.4)	51 (19.5)	1 (0.4–2.5)	
CCND2	rs10849004	C>T	CC	22 (75.9)	169 (67.3)	1	0.41
			CT	5 (17.2)	72 (28.7)	0.7 (0.3–1.6)	
			TT	2 (6.9)	10 (4)		
EBF1†	rs71593317	A>C	AA	29 (90.6)	202 (79.2)	1	0.13
			AC, CC	3 (9.4)	53 (20.8)	0.4 (0.1–1.4)	
GRID2†	rs34144324	C>T	CC	28 (93.3)	220 (92.4)	1	0.90
			CT, TT	2 (6.7)	18 (7.6)	0.9 (0.2–4.2)	
GRIK1	rs2154490	G>A	GG	18 (56.3)	156 (61.4)	1	0.57
			GA	13 (40.6)	83 (32.7)	1.2 (0.6–2.6)	
			AA	1 (3.1)	15 (5.9)		
GRIN3A†	rs10989692	G>A	GG	25 (78.1)	210 (78.1)	1	0.49
			GA, AA	7 (21.9)	43 (17)	1.4 (0.6–3.4)	
KCNMA1	rs11594258	A>T	AA	16 (51.6)	178 (68.7)	1	0.06
			AT	15 (48.4)	71 (27.4)	2.1 (1–4.3)	
			TT	0 (0)	10 (3.9)		
KLF12	rs1536407	C>A	CC	10 (31.3)	112 (46.5)	1	0.10
			CA	18 (56.3)	102 (42.3)	1.9 (0.9–4.2)	
			AA	4 (12.5)	27 (11.2)		
NARF	rs4789693	A>C	AA	16 (50)	144 (56.9)	1	0.46
			AC	16 (50)	90 (35.6)	1.3 (0.6–2.8)	
			CC	0 (0)	19 (7.5)		
PCSK5	rs11144550	G>A	GG	23 (71.9)	199 (78.3)	1	0.41
			GA	8 (25)	49 (19.3)	1.4 (0.6–3.2)	
			AA	1 (3.1)	6 (2.4)		
PROX1-AS1†	rs1891059	G>A	GG	27 (84.4)	227 (89.7)	1	0.37
			GA, AA	5 (15.6)	26 (10.3)	1.6 (0.6–4.6)	
SH3YL1†	rs4241316	G>A	TT	26 (83.9)	198 (81.5)	1	0.75
			TC, CC	5 (16.1)	45 (18.5)	0.9 (0.3–2.3)	
SOX14	rs6797178	G>A	GG	10 (31.3)	119 (45.4)	2.3 (1.1–5.2)	0.03*
			GA	22 (68.8)	112 (42.7)		
			AA	0 (0)	31 (11.8)	NA	

Three SNPs in DOK5, BMP7 and LINC00251 genes were excluded from the analyses due to the MAF lower than 5%. Frequencies for each genotype group are presented in affected (with osteonecrosis) and unaffected (without osteonecrosis) individuals, except for cases with low number of minor allele homozygotes, where carriers of minor allele are combined (marked by †). OR is calculated according to dominant or recessive models.

*Statistically significant at below $p < 0.05$.

†In cases with low number of minor allele homozygotes, where carriers of minor allele are combined.

Gen.: Genotype; NA: Not applicable (OR not calculated due to 0 in one of the cells); OR: Odds ratio; Subst.: Substitution.

administered to this group. The effect of genotype is presented by odds ratio (OR) with 95% CI according to the best model, which, in all cases, was either dominant or recessive. Unadjusted p-values were reported. False discovery rate (FDR) [23] was also calculated to account for multiple testing corrections. For the significantly associated variants, multivariable logistic regression analysis was also performed to assess the effect of genotype while controlling for nongenetic covariates, including age, sex, CS type, treatment protocol, leukemia cell type and risk groups. Statistical analyses were performed by SPSS statistical package (IL, USA), version 22.0. The Optimal Sequence Kernel Association test [24] was used in RStudio© software for rare variants associations.

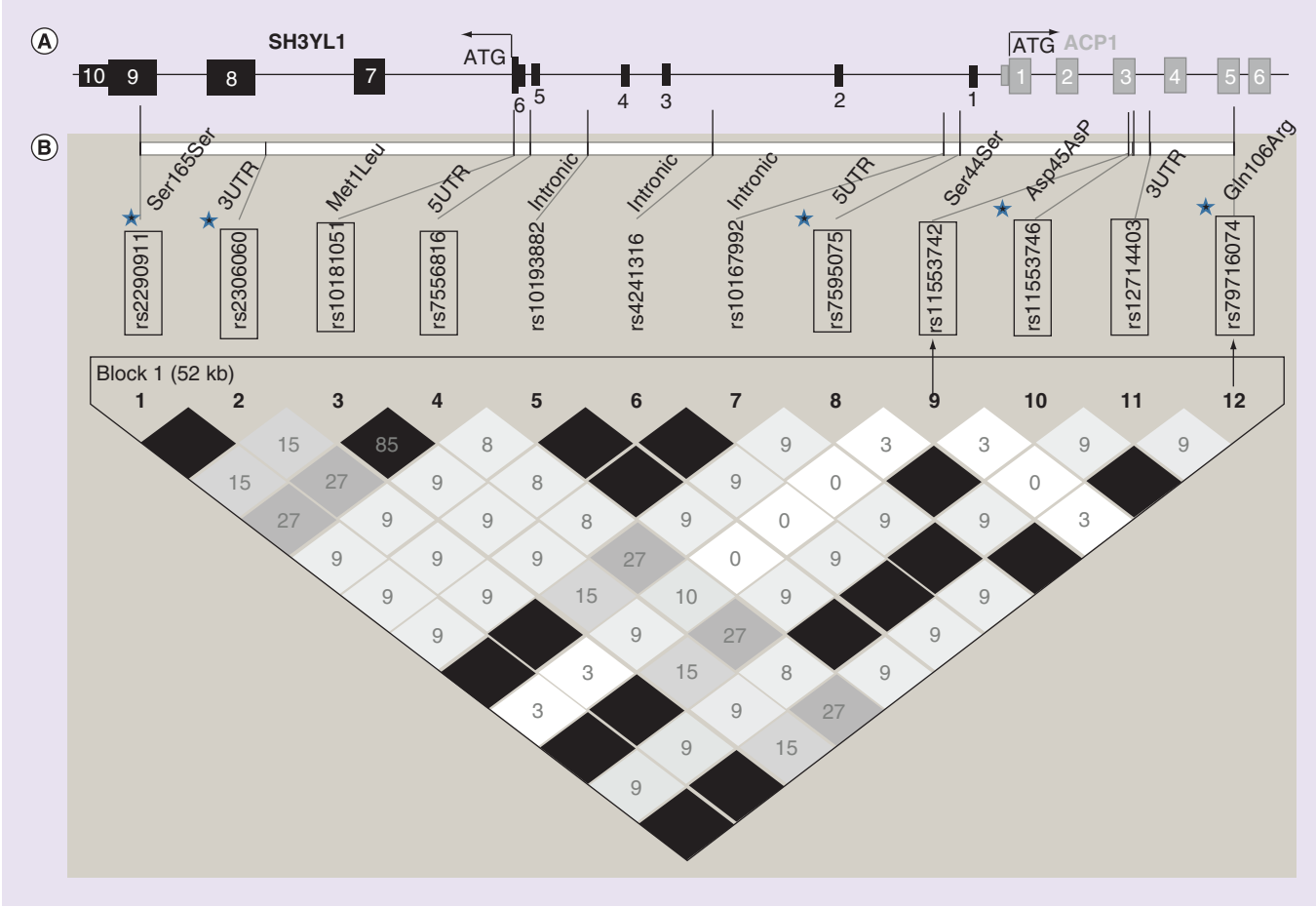

Figure 1. Schematic representation of *SH3YL1/ACP1* locus in A and pair-wise R^2 display of analyzed SNPs in *SH3YL1/ACP1* locus in B. (A) Large and small rectangles represent coding and noncoding exons (UTR) respectively. **(B)** SNPs of *SH3YL1-ACP1* locus, either reported previously as GWAS SNPs or SNPs derived from WES data (in black rectangle) of QcALL cohort, are depicted. Pair-wise R^2 display is obtained by Haploview. SNPs correlated with $R^2 \geq 0.8$ and associated with osteonecrosis are marked with an asterisk. SNPs defining *ACP1*A/*B/*C* alleles are marked with an arrow. Position in protein is given respective to NM_007099.3 mRNA.
GWAS: Genome-wide association studies; QcALL: Quebec childhood acute lymploblastic leukemia.

Results

Association analysis of 13 GWAS SNPs with ON (Table 1) revealed a significant association of one polymorphism, G>A substitution (rs6797178) in *SOX14* gene, according to the recessive model. Lower frequency of AA genotype was noted among affected patients as compared with nonaffected (0% vs. 11.8%; p = 0.03; Table 1). However, the association did not sustain correction for multiple testing. The analyses of 43 exonic variants in GWAS genes (Supplementary Table 4) identified in HR patients, significant associations of 3 SNPs in *ACP1/SHY3L1* locus, of which two remained significant after correction for multiple testing with FDR lower than 15% (rs2306060 and rs2290911). Associated SNP belong to the same linkage disequilibrium (LD) block in which rs2306060 and rs22909115 are in complete LD with 3 other variations (rs11553746, rs7595075 and rs79716074, Figure 1). All five SNPs were further genotyped in entire QcALL and all were associated with ON in HR patients according to the recessive model (Table 2). For example, the frequency of TT genotype of rs11553746 C>T was 35.7% versus 8.3% in cases with and without ON respectively (p = 0.009; Table 2), resulting in higher risk of ON for TT genotype (OR: 6.2; 95% CI: 1.8–21.6). The rs11553746 C>T polymorphism was also tested in multivariable models; TT genotype and the use of DXM were both associated with an increased risk of ON in patients assigned to HR group (OR: 9.1; 95% CI 2.1–39.1; p = 0.003, OR: 6.2; 95% CI: 1.6–24.3, p = 0.01 respectively; Table 3).

The rs11553746 was further genotyped in the replication cohort and association with ON was noted for *T* allele carriers when all patients of that cohort were analyzed (p = 0.04, Figure 2).

Table 2. Relationship of *ACP1* SNPs with osteonecrosis upon stratification to high-risk group.

Gene/SNP/position	Genotype	Affected	Nonaffected	OR	p-value
ACP1, rs11553746 Chr. 2:272203	CC	5 (35.7)	59 (44.4)	1	1.000
	CT	4 (28.6)	63 (47.4)	0.7 (0.2–2.9)	0.740
	TT	5 (35.7)	11 (8.3)	5.4 (1.3–21.7)	0.024*
	CC,CT	9 (64.3)	122 (91.7)	6.2 (1.8–21.6)	0.009*
	TT	5 (35.7)	11 (8.3)		
ACP1, rs79716074, Chr. 2: 277003	AA	5 (35.7)	61 (45.2)	1	1.000
	AG	4 (28.6)	62 (45.9)	0.8 (0.2–3.0)	1.000
	GG	5 (35.7)	12 (8.9)	5.1 (1.3–20.3)	0.027*
	AA,AG	9 (64.3)	123 (91.1)	1	1.000
	GG	5 (35.7)	12 (8.9)	5.7 (1.6–19.7)	0.011*
SH3YL1, rs2290911, Chr. 2: 224919	AA	5 (35.7)	57 (43.2)	1	1.000
	AG	4 (28.6)	65 (49.2)	0.7 (0.2–2.7)	0.735
	GG	5 (35.7)	10 (7.6)	5.7 (1.4–23.3)	0.020*
	AA,AG	9 (64.3)	122 (92.4)	1	1.000
	GG	5 (35.7)	10 (7.6)	6.8 (1.9–24.1)	0.007*
SH3YL1, rs7595075, Chr. 2: 264019	CC	5 (35.7)	62 (46.6)	1	1.000
	CA	4 (28.6)	61 (45.9)	0.8 (0.2–3.2)	1.000
	AA	5 (35.7)	10 (7.5)	6.2 (1.5–25.3)	0.016*
	CC,CA	9 (64.3)	123 (92.5)	1	1.000
	AA	5 (35.7)	10 (7.5)	6.8 (1.9–24.3)	0.006*
SH3YL1, rs2306060, Chr. 2: 230912	AA	5 (35.7)	58 (43.9)	1	1.000
	AC	4 (28.6)	65 (49.2)	0.7 (0.2–2.8)	0.736
	CC	5 (35.7)	9 (6.8)	6.4 (1.6–26.8)	0.015*
	AA,AC	9 (64.3)	123 (93.2)	1	1.000
	CC	5 (35.7)	9 (6.8)	7.6 (2.1–27.5)	0.005*

*Statistically significant at below $p < 0.05$.
Physical location of SNP based on February 2009 (GRCh37/hg19).

Table 3. Risk of osteonecrosis in Quebec childhood acute lymploblastic leukemia patients associated with *ACP1 rs11553746 C>T* polymorphism in multivariable logistic regression model.

Categorical variable	p-value	OR (95% CI)
ACP1 rs11553746	0.003	9.1 (2.1–39.1)
Corticosteroid	0.010	6.2 (1.6–24.3)
Protocol	0.878	0.9 (0.5–1.9)
Age	0.202	2.3 (0.6–8.5)
Sex	0.541	1.5 (0.4–5.1)
Cell phenotype	0.061	3.7 (0.9–14.2)

The genotype and covariates are recoded as categorical variables, *ACP1* rs11553746 is either TT (1) or remaining genotypes (0), age is ≥10 (1) or <10 years (0), corticosteroid is dexamethasone (1) or prednisone (0), cell phenotype is B- (0) or T-cell leukemia (1); and for sex, girls are 1 and boys 0. DFCI protocols (87-01, 91-01, 95-01 and 00-01) are recoded from 1 to 4 starting from the most recent protocol.

Among five SNPs in LD which were associated with ON, the G allele of the rs79716074 *A>G* defines haplotype **B* of functional *ACP1*A/*B/*C* alleles (Figure 3) [25]. Given that the *GG* genotype of this SNP was associated with ON (Table 2), there was also an association of the resulting **B*B* diplotype,

The rs11553746 (and other related SNPs) were not in complete LD with previously identified GWAS SNPs in *ACP1/SHY3L1* (Figure 1) [16]. The rs11553746 (and other SNPs in LD) or occurrence of ON did not correlate with event free survival (data not shown).

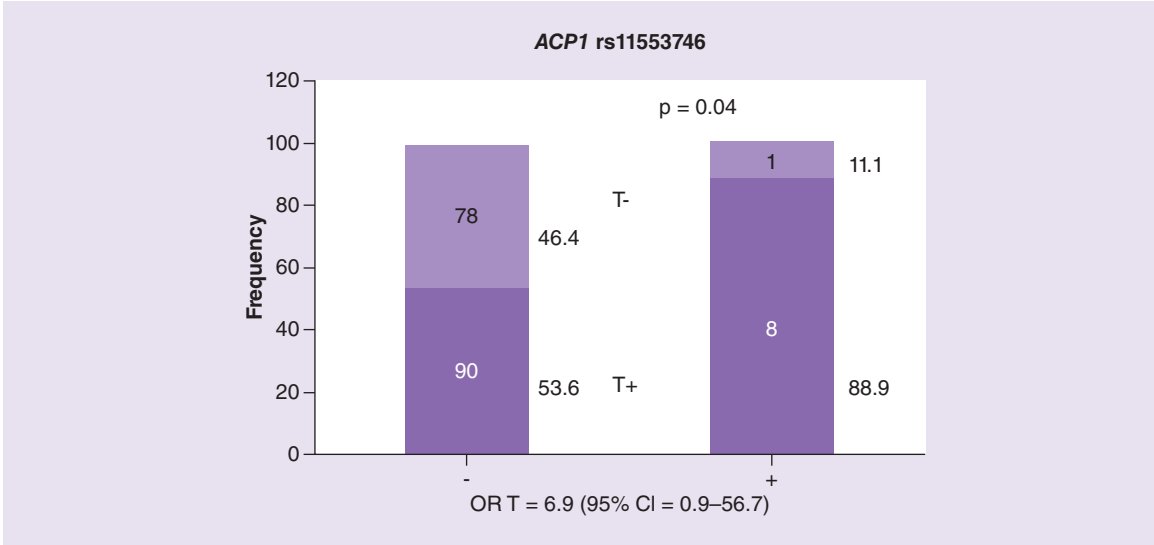

Figure 2. The frequency of *ACP1* rs11553746 in replication cohort. Cases with and without osteonecrosis are each represented with the bar and within each bar, frequency of cases with and without *T* allele are depicted in black and grey color respectively. The exact frequency and the number of patients in each subgroup are indicated; p-value for the difference between genotype groups and odds ratio with 95% CI are given below plots.

	rs11553742Ser44Ser (Exon 3)	rs79716074Gln106Arg (Exon 5)	
*A	C	A	58
*B	C	G	35
*C	T	A	7

Figure 3. Relationship between SNPs and *ACP1*A/*B/*C* nomenclature. rs11553742 splicing variant and rs79716074 missense variant defines *ACP1*A/*B/*C* alleles. rs79716074 defines allele **B* and is one of the SNPs in linkage disequilibrium associated with osteonecrosis in this study. Frequency of each allele in Quebec childhood acute lymploblastic leukemia cohort is indicated.

Analysis of rare variants in GWAS genes identified association of collapsed variants in *SOX14* and *EBF1* genes (p = 0.03 and 0.02 respectively; Supplementary Table 5). However, in both cases, FDR was higher than 15% and they were not further analyzed in the replication group.

Discussion

Here we investigated previously reported top-ranking GWAS *loci* of ON as well as other variants located in the same genes for their influence on development of this complication in childhood ALL patients treated with DFCI protocols. Although originally reported GWAS SNPs in *ACP1* gene (and in *SHY3L1* in close proximity) were not replicated, several variants in the same locus were associated with ON. The association was maintained in replication cohort. Among five SNPs in LD, two are interesting from functional perspective; the rs11553746 C>T, which depending on mRNA isoform, is either synonymous (Asp45Asp) or missense leading to *ACP1* Thr95Ile replacement and the rs79716074 A>G variation that defines **B* haplotype of *ACP1* gene. *ACP1* controls the synthesis of cytosolic low molecular weight protein tyrosine phosphatase [26], which is a key regulator in many cellular processes, including regulation of growth factors and cellular adhesion [25,27,28]. Two different isoenzymes that arise through alternative splicing have been described and based on electrophoretic mobility are defined as 'fast' (F) and 'slow' (S). Alternative splicing is influenced by the presence of 3 codominant **A, *B* and **C* alleles [29]. The rs79716074, which defines allele **B*, is a missense Glu106Arg variant, whereas rs11553742, defining allele **C*,

alters motif recognized by splicing factors SRp40 and SRp55 [30]. Allele **B* and **C* are associated with higher levels of F and S isoform, respectively [25]. *ACP1* alleles have been associated with susceptibility to several diseases, such as inflammatory and autoimmune diseases, cancer growth and metastatic bone cancer [26,31]. The pathogenesis of CS-induced bone disease (in ALL patients and beyond) is thought to arise from an increased apoptosis of osteoblasts and osteocytes and prolongation of the osteoclasts lifespan [32]. CS excess causes overproduction of reactive oxygen species [32] and may modulate vascular responsiveness to vasoactive substances leading to apoptosis of endothelial cells. CS were also reported to elevate the level of adipogenesis, leading to fat hypertrophy in the bone marrow [32]. *ACP1* contribution to ON can be linked to its role as an important signaling molecule in osteoblast biology and bone formation [33] and its expression/activity, which is modulated during osteoblastic differentiation in response to the redox status of the cells [33]. In regard to *ACP1* isoforms, it has been shown that *ACP1* increases osteoclastic differentiation likely at the expense of the F isoform [34], whose highest concentration was reported for **B* allele [25]. F isoform may also have a specific role in the regulation of quantity of adipose tissue [35], whereby higher F levels were associated with higher risk of hypertriglyceridemia and high cholesterol levels [36].

In conclusion, we confirmed the implication of *ACP1* locus in the development of ON in childhood ALL patients. We did not confirm the association of the same SNP(s) in this gene, as in original GWAS of ON in childhood ALL [16]. This might be due to difference in population studied (GWAS was performed in population of mixed ancestry), reflected by different minor allele frequency and resulting LD pattern, difference in the treatment protocol relative to corticosteroid type, cumulative dose or administration schedule and limited sample size of our study. These differences along with the wide range of SNPs might have also contributed to the lack of association noted for other studied genes. Possible implication (not maintained after corrections for multiple testing) was nevertheless noted for *SOX14* and *EBF1* genes, which warrant further attention.

This study identified potential causal SNP in *ACP1* gene that can through the influence on F isoform level and resulting pathophysiological mechanisms, contribute to the development of ON, at least in patients of European ancestry treated with DFCI protocols.

Summary points

- Osteonecrosis (ON) is a dose-limiting corticosteroid (CS)-related complication, reported in children with acute lymphoblastic leukemia (ALL).
- To detect the genetic component underlying the risk of ON, which may lead to personalized treatment or prophylactic options, several genome-wide association studies have been conducted.
- Lead SNPs from these studies and other variants in the same genes, which were pooled from whole exome sequencing data, were analyzed for an association with ON in children with ALL treated with DFCI protocols.
- The analyses in discovery and replication cohorts identified previously undetected variants in the *ACP1* gene as a predictor of ON, including rs11553746 C>T with OR: 6.2; 95% CI: 1.8–21.6 ($p = 0.009$).
- The *11553746 C>T* is in complete linkage disequilibrium with several SNPs (rs2290911, rs7595075, rs79716074 and rs2306060) in the *ACP1/SHY3L1* locus; the rs79716074 in *ACP1* gene is particularly interesting as it results in amino-acid replacement and underlines **B* haplotype of *APC1,* which is associated with higher level of functional F isoform.
- ACP1 is an important signaling molecule in osteoblast biology and bone formation.
- *ACP1* gene variant is a potential marker for CS-related bone morbidity in childhood ALL patients.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/pgs-2019-0087

Author contributions

M Krajinovic designed the study; M Plesa, V Gagné, A Aubry-Morin and R Abaji performed experiments; M Plesa performed medical chart reviews; C Laverdière, JM Leclerc, N Alos, SE Sallan, D Neuberg, JL Kutok, LB Silverman and D Sinnett contributed to sample, sequencing and clinical data collection and interpretation; Beaulieu P and St-Onge P performed bioinformatics analyses. V Gagné, A Aubry-Morin, K Petrykey and M Krajinovic performed the data analysis; M Krajinovic drafted the article; all authors contributed to the interpretation of data and revised the manuscript.

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Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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