Genetic risk factors for the development of osteonecrosis in children under age 10 treated for acute lymphoblastic leukemia


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Running Title: Genetic risks of osteonecrosis in young children

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1. Variants in genes important for MSC differentiation influence the risk of osteonecrosis in children with ALL under 10 years old.
2. Variants in genes in the glutamate signaling pathway influence osteonecrosis in children with ALL regardless of age.

Abstract

Therapy related osteonecrosis has become a dose-limiting toxicity in the treatment of pediatric acute lymphoblastic leukemia (ALL). Prior studies on the genetic determinants of osteonecrosis have focused on patients 10 years and older, leaving the genetic risk factors for the larger group of children younger than 10 years incompletely understood.

Here, we perform the first evaluation of genetic risk factors for osteonecrosis in children less than 10 years old. The discovery cohort comprised 82 cases of osteonecrosis and 287 controls treated on Children’s Oncology Group (COG) standard-risk ALL protocol AALL0331 (NCT00103285, https://clinicaltrials.gov/ct2/show/NCT00103285), with results tested for replication in 817 children less than 10 years treated on COG high-risk ALL protocol AALL0232 (NCT00075725, https://clinicaltrials.gov/ct2/show/NCT00075725).

The top replicated SNPs were located near bone morphogenic protein 7 [BMP7: rs79085477 and rs75161997, P=5.34x10^{-8} (OR 15.0) and P=0.0498 (OR 8.44) in the discovery and replication cohorts, respectively] and PROX1-antisense RNA1 [PROX1-AS1: rs1891059, P=2.28x10^{-7} (OR 6.48) and P=0.0077 (OR 3.78) for the discovery and replication cohorts, respectively]. The top
replicated non-synonymous SNP, rs34144324, was in a glutamate receptor gene \([GRID2, P=8.65 \times 10^{-6} \text{ (OR 3.46)} \text{ and } P=0.0136 \text{ (OR 10.8)}\) in the discovery and replication cohorts, respectively]. In a meta-analysis, the replicated \(BMP7\) and \(PROX1-AS1\) variants (rs75161997 and rs1891059, respectively) met the genome-wide significance threshold of \(<5 \times 10^{-8}\). Top replicated SNPs were enriched in enhancers active in mesenchymal stem cells, and pathway analysis of annotated genes demonstrated enrichment in glutamate receptor signaling and adipogenesis pathways. These data may provide new insights into the pathophysiology of osteonecrosis.

**Introduction**

Progressive intensification of multi-agent chemotherapy has improved outcomes for children with acute lymphoblastic leukemia (ALL), with cure rates exceeding 90%.\(^1^\)\(^6\) Results have been particularly favorable for patients less than 10 years of age with National Cancer Institute (NCI) standard-risk (SR) ALL.\(^7^\)\(^8\) Unfortunately, such intensified therapy has also been associated with significant increases in the occurrence of therapy related osteonecrosis.\(^9^\)\(^1^\)\(^2\)

Prior studies have identified multiple clinical risk factors for the development of osteonecrosis, including female gender, European ancestry, the administration of 3 weeks continuous rather than alternate-week dexamethasone during delayed intensification, and intensive vs. standard therapy.\(^9^\)\(^1^\)\(^1\) However, age remains the strongest and most consistently identified factor, with patients 10-20 years old at greatest risk.\(^1^\)\(^0^\)\(^1^\)\(^1\)\(^3^\)\(^1^\)\(^5\)\(^1^\)

Given this age-related risk, the majority of children in prior investigations of genetic predisposition to osteonecrosis were older than 10 years.\(^1^\)\(^0^\)\(^1^\)\(^5\) However, because ALL is so
common in young children, up to 40% of osteonecrosis cases develop in children under 10 years.10

In this study, we identified genetic factors associated with the development of osteonecrosis in the largest cohort of SR ALL patients evaluated to date, and validated these findings in a cohort of NCI high-risk (HR) ALL patients less than 10 years of age.

**Methods**

**Patients**

The discovery cohort consisted of children with newly diagnosed SR B-precursor ALL treated on Children’s Oncology Group (COG) AALL0331 (NCT00103285) with germline DNA available; of these, all patients with osteonecrosis as of 6/30/2012 were submitted for genotyping as cases. Controls were taken from a previously genotyped sub-cohort of AALL0331 (Supplemental Methods). Of 111 cases who were evaluable post-induction and developed grade 2-4 osteonecrosis, genotyping was completed for 96. Patients (N=34, 14 cases and 20 controls) who received alternate-week dexamethasone after protocol amendment 2C (see supplement for details) were excluded from the analysis (Figure 1). Controls were excluded if less than 1000 days of follow-up data were available, a time when the majority of osteonecrosis was detected. Following these steps, the discovery cohort included 82 osteonecrosis cases and 287 controls (Figure 1).

The replication cohort consisted of children less than 10 years of age at diagnosis treated for newly diagnosed HR B-precursor ALL on COG AALL0232 (NCT00137111). The
characteristics of this cohort have been previously described.\textsuperscript{15} The analysis was limited to the 817 children (20 cases, 797 controls) with genotype and phenotype data available (Supplemental Figure 1).

Informed consent was obtained from patients 18 years and above, and from parents or guardians of patients under 18 years in accordance with the Declaration of Helsinki. The COG AALL0331 and AALL0232 protocols were approved by the National Cancer Institute and the institutional review boards of participating institutions.

Patients on both protocols were prospectively monitored for clinical signs and symptoms of osteonecrosis and the diagnosis confirmed by imaging according to institutional preference. Osteonecrosis was considered a reportable event and was graded using the National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.0; controls had absent (grade 0) or asymptomatic (grade 1) osteonecrosis; cases had moderate (grade 2), severe (grade 3), or disabling (grade 4) osteonecrosis.

**Genotyping**

Genotyping of single nucleotide polymorphisms (SNPs) for AALL0331 and AALL0232 was performed using the Illumina Human Exome BeadChip v1.1 and the Affymetrix Gene Chip Human Mapping Array 6.0. For SNPs not interrogated by direct genotyping, genotypes were imputed using MaCH-Admix software (University of North Carolina), using phase 1 release v3 of the 1000 Genomes Project (http://www.1000genomes.org) as the reference genomes.\textsuperscript{16}

For a subset of 15 cases of osteonecrosis, whole exome sequencing was performed. Library generation was performed using NimbleGen SeqCap EZ Exome Enrichment Kit v2.0, and sequencing was performed using Illumina HiSeq 2000. Raw reads were aligned against human
genome hg19 using Burrow-Wheeler Aligner.\textsuperscript{17} Raw reads were converted to genotypes using the Genome Analysis Toolkit (GATK) version 3.2.\textsuperscript{18}

\textit{Definition of genetic ancestry}

Genetic ancestry for all patients was defined using STRUCTURE v2.2.3 as previously described.\textsuperscript{19} When ancestry was assessed as a categorical variable, individuals were classified as white, black, or Hispanic based on percentage inferred genetic ancestry as follows: >90% Northern European (CEU) were classified as white; >70% West African (YRI) classified as black; those with Native American ancestry >10% and greater than the % West African ancestry were classified as Hispanic. Patients not falling into these groups were categorized as Other.

\textit{Quality control}

All SNPs with a call rate of <95% or poor clustering were excluded. SNPs were excluded if they deviated from Hardy-Weinberg equilibrium in whites ($P<1\times 10^{-4}$). SNPs with a minor allele frequency (MAF) >5%, and SNPs with an MAF >0.5% with the minor allele as the risk allele for the development of osteonecrosis, were included.

\textit{Statistical analysis}

AALL0331 treatment arms were clustered into therapy groups based on pre-maintenance therapy duration, dexamethasone and asparaginase dosing, and previously reported therapy groups at increased risk for the development of osteonecrosis (Supplemental Methods, Supplemental Table 1).\textsuperscript{20} Within the discovery cohort, SNP genotypes were compared between cases and controls while controlling for age, genetic ancestry as continuous variables, gender, and therapy group. Evaluation used a time-to-event proportional hazard regression analysis with patients censored at
the time of diagnosis of osteonecrosis or at last follow-up. Relapse and off-protocol therapy were treated as competing events. Analysis was performed using the R survival package (v2.38-1) implemented in PLINK (v1.07; http://pngu.mgh.harvard.edu/purcell/plink/) using Rserve.\textsuperscript{21,22} Additional data analysis was performed using R (v3.1.1; www.r-project.org).\textsuperscript{23}

To test for validation, a parallel GWAS was performed in the replication cohort. SNP alleles identified in the discovery cohort were considered validated if the $P$ values in both cohorts were $<.05$ (Supplemental Figure 2). A meta-analysis was performed for all SNPs passing quality control in both the discovery and replication cohorts using Stouffer’s Z-score method weighted by the GWAS standard error of the SNP in each cohort.\textsuperscript{24}

Enhancer enrichment analysis was performed using HaploReg (v2).\textsuperscript{25} Validated SNPs with a meta-analysis $P<.001$ were evaluated for enhancer enrichment across available Roadmap samples. Associations were sorted according to their enrichment for enhancers and strongest enhancers.\textsuperscript{26}

SNPs validated in both cohorts with a meta-analysis $P<.001$ were annotated to genes, with the closest gene selected for intergenic SNPs. Pathway analysis was performed using QIAGEN Ingenuity Pathway Analysis (IPA; www.qiagen.com/ingenuity) to identify canonical biological pathways enriched within top SNPs. Only pathways with at least 5 genes associated with osteonecrosis were considered.

**Results**

**GWAS for osteonecrosis**
We identified covariates to include in the GWAS for osteonecrosis in the discovery cohort (Supplemental Table 2) which included age ($P=1.88 \times 10^{-10}$), therapy group ($P=6.8 \times 10^{-4}$ for the difference across all 4 groups), gender ($P=.008$), and genetic ancestry ($P=.02$ for European ancestry).

A GWAS of the discovery cohort adjusting for the covariates of age, gender, therapy group, and ancestry (Supplemental Table 2) identified 16 SNPs in six genes associated with osteonecrosis at a $P$ value threshold of $<5 \times 10^{-8}$ (Supplemental Table 3). Two SNPs, rs77556622 and rs76599360 (both at $P=1.13 \times 10^{-9}$) were located ~11kb and 20.7kb, respectively, 3’ of BMP7 (bone morphogenic protein 7). The risk alleles (C and T, respectively) for both SNPs were present in 5 of 82 cases and none of the 287 controls and conferred a 22 fold increase in osteonecrosis risk (95% confidence interval (CI) 8.15-59.6). Patients with these SNPs developed osteonecrosis despite clinical characteristics placing them at low risk, including three patients in the lowest risk treatment group, two males, and one patient with black ancestry (Figure 2).

In the replication cohort, 10 of the top 100 SNPs from the discovery cohort were validated, including 2 SNPs (rs79085477, rs75161997) in BMP7 in full LD with one another, with $P=5.34 \times 10^{-8}$ (HR 15.0, 95% CI 5.64-39.7) in the discovery cohort and .0498 (HR 8.43, 95% CI 1.002-71.1) in the replication cohort. The top replicated SNPs represented 5 loci across 4 genes including BMP7 (2 SNPs), PROX1-AS1 (6 SNPs), LINC00251 (1 SNP), and DOK5 (1 SNP) (Table 1). Interestingly, the top validated SNPs in both BMP7 and PROX1-AS1 (rs7516997 and rs1891059, respectively) are predicted to increase glucocorticoid receptor binding based on binding site motif alterations.
Meta-analysis

Meta-analysis of both cohorts identified 5 loci with genome-wide significant associations with osteonecrosis \((P<5\times10^{-8})\), 3 of which were significant in both cohorts, including loci near PROX1-AS1 and BMP7 (Table 1, Figure 3). The top SNP from the meta-analysis (rs9632544 near ERV3-I) was significant in the discovery but not the replication cohort \((P=2.36\times10^{-9} \text{ and } P=.052, \text{ respectively})\).

In the meta-analysis, the top replicated coding SNP was rs34144324, a missense variant in GRID2 (glutamate receptor, ionotropic, delta 2) with a meta-analysis \(P=7.07\times10^{-7}\) (HR 3.78, 95% confidence interval 2.23-6.39; \(P=8.65\times10^{-6}\) and \(P=.014\) in the discovery and replication cohorts, respectively). In the 15 patients with whole exome sequencing available, 12 non-synonymous variants were sequenced with a \(P<.001\) in the meta-analysis. There was 99.4% agreement in variant calls between the array-based genotype calls and the sequencing genotype calls, including 100% agreement for the GRID2 SNP.

Enhancer enrichment analysis

SNPs validated in both cohorts with a meta-analysis \(P<.001\) (N=3,271) were evaluated for enhancer enrichment using HaploReg. We focused on tissues in which SNPs were enriched in both all enhancers and the strongest enhancers: colon smooth muscle, adipose derived mesenchymal stem cells, bone marrow derived mesenchymal stem cells, and duodenal smooth muscle. When restricting the analysis to validated SNPs with a meta-analysis \(P <1\times10^{-5}\) (N=140, Supplemental Table 4), enhancer enrichment was observed only in bone marrow derived mesenchymal stem cells [2 fold enrichment for all enhancers \((P=.0148)\) and 4.3 fold enrichment for the strongest enhancers \((P=2.81\times10^{-4})\)]. Adipose derived mesenchymal stem cells remained
enriched for SNPs in all enhancers (1.8 fold, \(P=.011\)) with marginal enrichment in the strongest enhancers (1.9 fold, \(P=.068\), Table 3).

**Pathway analysis**

There were 3,271 SNPs with meta-analysis \(P<.001\) and significant associations with osteonecrosis \((P<.05)\) in both AALL0331 and AALL0232 cohorts (the top 140 with \(P<1\times10^{-5}\) are shown in Supplemental Table 4). The 459 genes to which these SNPs annotated were significantly enriched within eight canonical pathways. Of these, seven contained overlapping glutamate receptor genes, with the glutamate receptor signaling pathway most overrepresented \((P=9.92\times10^{-4}, 10.5\% \text{ of genes in pathway associated with osteonecrosis})\). The adipogenesis pathway was the only overrepresented pathway \((P=.0148, 5.5\% \text{ of genes in pathway associated with osteonecrosis})\) whose genes did not overlap with glutamate receptor signaling (Table 2, Supplemental Table 5).

**Discussion**

Osteonecrosis has been recognized in recent years as a major toxicity of pediatric ALL therapy. Children under 10 constitute approximately 75\% of pediatric ALL cases.\(^{27}\) Thus, while osteonecrosis incidence is low in this age group, those under 10 constitute up to 40\% of cases of osteonecrosis.\(^{10}\) It is hypothesized that inherited risk factors may be more penetrant in young rather than older patients. Better understanding of the individual risk factors for the development of osteonecrosis in younger children and the underlying biology in this population and in older patients is needed to improve prediction and management of this often debilitating complication.
These patients who developed ON despite being at low risk based upon age and treatment factors may provide unique insights into the genetic variations that influence development of ON.

The occurrence of osteonecrosis is highly dependent on the therapeutic regimen that patients receive. Thus, the identification of genetic variants that increase osteonecrosis risk in patients under 10 years of age treated on both standard (AALL0331)- and high-risk therapy (AALL0232) protocols suggests the identified variants may be important to osteonecrosis risk in this age group in the context of any “modern” ALL protocol (utilizing both minimal residual disease directed intensification of therapy, PEG-asparaginase, and a delayed intensification phase).

We performed the first GWAS for osteonecrosis risk in patients exclusively less than age 10 years and identified novel risk variants near BMP7 and PROX1-AS1 (Table 1). Studies have shown that BMP7 is released in response to bone damage in osteoarthritis and spondyloarthritis, and its release is increased by mechanical stress. Bone morphogenic proteins can induce mesenchymal precursor cells to differentiate into osteoblasts and inhibit the formation of osteoclasts, and thus variants affecting this gene could contribute to osteonecrosis by altering bone metabolism and formation both prior to and during ALL therapy. Moreover, BMP7 is known to be toxic to vascular smooth muscle. The prominent role of local arteriopathy, at least in part due to vascular smooth muscle damage, in the development of osteonecrosis supports the possibility that BMP7 could contribute to osteonecrosis via direct toxic effects on local bone vasculature.

PROX1 has been shown to control the differentiation of lymphatic endothelial cells from vascular endothelial cells. PROX1 was also noted to be down-regulated in familial combined hyperlipidemia, and it has been argued that this results in reduced clearance of plasma lipids in
this disease.\textsuperscript{37} It is possible that the identified variants in PROX1-AS1 increase the risk of osteonecrosis by altering lipid trafficking either within the compartment of the bone marrow or by increasing plasma lipids, a previously identified risk factor.\textsuperscript{10}

The importance of the identified SNPs in osteonecrosis is further supported by the findings of the enhancer enrichment analysis. The top 92 validated SNPs were enriched for locations within enhancers active in tissues closely related to osteonecrosis, specifically mesenchymal progenitors (Table 3). These cells, under the influence of cytokines including BMP7\textsuperscript{38} and environmental signals including glucocorticoids,\textsuperscript{39} vitamin D and mechanical load,\textsuperscript{29} can differentiate into either osteoblasts or adipocytes.\textsuperscript{40,41} SNPs significantly associated with osteonecrosis ($P<.001$) were linked to seven genes in the adipogenesis pathway (Table 2), supporting the importance of genes affecting mesenchymal differentiation in osteonecrosis. These findings are additionally supported by the findings in prior GWAS linking variants in fat and cholesterol metabolism genes (including ACP1) with osteonecrosis.\textsuperscript{10}

Pathway analysis demonstrated that variation in glutamate receptor signaling contributes to osteonecrosis in children under 10 years. Variants in six genes in the glutamate receptor pathway were associated with the development of osteonecrosis in this cohort, including the top validated non-synonymous variant (rs34144324 in GRID2, Table 2). Interestingly, the glutamate receptor signaling pathway was the top pathway represented by genetic variants in a cohort of HR patients of all ages.\textsuperscript{15} While glutamate receptor signaling appears to play a role in osteonecrosis across the age spectrum, the association appears stronger in older patients than in young patients, as both the top SNP and top pathway in a cohort dominated by older patients were related to glutamate signaling.\textsuperscript{15} In contrast, in young patients genetic variation in genes expressed in mesenchymal and adipose tissues appear more important as supported by single SNP, enhancer,
and pathway analyses (Tables 1-3). It is also possible that the apparent stronger association of glutamate receptor signaling in the prior study reflects the effects of high-risk therapy in addition to differences in age. However, prior findings in the Vanderbilt BioVU cohort, where most patients were over 10 years old, and who developed osteonecrosis after glucocorticoids outside the treatment of ALL,\textsuperscript{15} suggests this association may be more due to age than therapy intensity.

Prior genome-wide studies\textsuperscript{10,15} failed to identify an association between osteonecrosis and \textit{BMP7}. This is likely due to the greater number of older patients in prior genome-wide studies. The reasons for the specificity of \textit{BMP7} to osteonecrosis risk in younger patients but not older patients are not clear. This may be explained by interactions between \textit{BMP7} and vitamin D, as vitamin D levels vary across the pediatric age range.\textsuperscript{42,43} \textit{BMP7} expression in the absence of vitamin D induces osteoblast differentiation and mineralization, but this effect is reversed in the presence of 1-25(OH)\textsubscript{2}-vitamin D\textsubscript{3}.\textsuperscript{44} Thus, it is possible that variation in \textit{BMP7} is important for osteonecrosis risk in younger but not older children due to age related differences in vitamin D levels. Alternatively, because \textit{BMP7} can induce angiogenesis,\textsuperscript{45,46} it is possible variants in \textit{BMP7} have a smaller impact on osteonecrosis in older children and adolescents when bony growth is complete and the bony vasculature is already established.\textsuperscript{15} The \textit{BMP7} and \textit{PROX1-AS1} variants replicated in AALL0232 patients less than 10 years of age ($P=0.0498$ and $P=0.008$, respectively) but not in patients age 10 or older ($P=0.73$ and $P=0.74$, respectively), which also suggests the effects of the variants are age rather than treatment specific. Future evaluation of these findings in additional standard- and high-risk patient populations under age 10 will allow further understanding of the interaction of younger age and treatment intensity in the development of osteonecrosis.
Conclusions

While less common in children under 10 years, osteonecrosis remains a major therapeutic toxicity in this age group. The findings of this GWAS provide new insight into the genetic features associated with the development of osteonecrosis in younger patients, while verifying that glutamate receptor genetic variation may be important across all age groups. Our findings support the notion that some genetic risk factors for osteonecrosis are age-specific whereas others are not.

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Authorship: Contributions: Concept and design: EAR and MVR. Collection and assembly of data: SEK, LAM, WY, KWM, CS, CL, TYC, MLL, SPH, MD, MVR. Analysis and interpretation of data: SEK, WY, CS, TYC, CC, MVR. All authors contributed to the writing of the manuscript.

Conflict of interest disclosure: The authors have no conflicts to disclose.

References


Table 1: Top single nucleotide polymorphisms from discovery cohort validated in replication cohort

<table>
<thead>
<tr>
<th>RSID</th>
<th>Gene</th>
<th>Risk allele</th>
<th>Discovery MAF cases</th>
<th>Discovery MAF controls</th>
<th>Discovery cohort $P^2$</th>
<th>Discovery cohort HR (95% CI)</th>
<th>Replication cohort P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs79085477</td>
<td>BMP7</td>
<td>T</td>
<td>0.03</td>
<td>0</td>
<td>5.34x10^-8</td>
<td>15.0 (5.64-39.7)</td>
<td>.0498</td>
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<td>rs75161997</td>
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<td>T</td>
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<tr>
<td>rs1891059</td>
<td>PROX1-AS1</td>
<td>A</td>
<td>0.061</td>
<td>0.017</td>
<td>2.28x10^-7</td>
<td>6.48 (3.19-13.2)</td>
<td>.008</td>
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<tr>
<td>rs74533616</td>
<td>LINCO00251</td>
<td>G</td>
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<td>0.002</td>
<td>8.48x10^-7</td>
<td>23.3 (6.65-81.5)</td>
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<td>LINCO00251</td>
<td>G</td>
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<td>0.018</td>
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<td>1.71x10^-6</td>
<td>21.0 (6.03-72.9)</td>
<td>.003</td>
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</table>

MAF: minor allele frequency; HR: hazard ratio; CI: confidence interval

1: $P$ value for association of SNP genotype with osteonecrosis risk in discovery cohort (N= 369)

2: $P$ value for association of SNP genotype with osteonecrosis in the replication cohort (N=817)
Table 2: Top non-overlapping pathways enriched in single nucleotide polymorphisms associated with osteonecrosis (meta-analysis $P<.001$) in combined discovery and replication cohorts (1186 total patients)

<table>
<thead>
<tr>
<th>Pathway (P for enrichment)</th>
<th>Gene</th>
<th>Top SNP(s) tagged to gene*</th>
<th>Meta-analysis P value of top SNP(s)</th>
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</thead>
<tbody>
<tr>
<td>Glutamate Receptor Signaling ($P=9.91 \times 10^{-4}$)</td>
<td>GRID2</td>
<td>rs34144324</td>
<td>7.07x10^{-7}</td>
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<tr>
<td></td>
<td>GRM5</td>
<td>rs308894</td>
<td>1.09x10^{-4}</td>
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<tr>
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<td>GRM7</td>
<td>rs112943394, rs112050403</td>
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<td>GRIN2B</td>
<td>rs1805491</td>
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<td>GNG7</td>
<td>rs112943394, rs112050403</td>
<td>9.74x10^{-4}</td>
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<td></td>
<td>SLC17A6</td>
<td>rs7127241</td>
<td>4.79x10^{-4}</td>
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<td>Adipogenesis ($P=.015$)</td>
<td>BMP7</td>
<td>rs79085477, rs75161997</td>
<td>8.28x10^{-9}</td>
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<td></td>
<td>EBF1</td>
<td>rs71593317</td>
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<td>NR1D2</td>
<td>rs62253386, rs62253388</td>
<td>4.07x10^{-5}</td>
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<td>CTBP1</td>
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<td></td>
<td>BMP2</td>
<td>rs117896134</td>
<td>3.16x10^{-4}</td>
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</table>

* Multiple SNPs in full LD are shown if all have equal associations with osteonecrosis

SNP: single nucleotide polymorphism
Table 3: Top validated single nucleotide polymorphisms (meta-analysis $P<1x10^{-5}$) are enriched in enhancers active in mesenchymal stem cells

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>All enhancers</th>
<th>Strongest enhancers*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fold enrichment</td>
<td>$P$</td>
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<tr>
<td>Bone marrow derived mesenchymal stem cell cultured cells</td>
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<tr>
<td>Adipose derived mesenchymal stem cell cultured cells</td>
<td>1.8</td>
<td>.011</td>
</tr>
</tbody>
</table>

*Strongest enhancer chromatin state as previously defined

Figure Legends

Figure 1: Consort diagram of 369 patients in discovery cohort treated on AALL0331.

Figure 2: Cumulative incidence of osteonecrosis based on genotype for top BMP7 variants.

All 5 patients in the discovery cohort (82 cases, 287 controls) carrying variant alleles of 2 SNPs in high LD [rs77556622 (T>C), rs76599360 (C>T); $P=1.13x10^{-9}$] located 3' to BMP7 developed osteonecrosis. Patient age, ancestry, gender, and therapy group are shown next to each case of osteonecrosis in the variant allele group. Therapy groups are defined within the supplemental materials, with group 1 receiving the most intense therapy and group 4 the least intense.

Figure 3: Manhattan plot of meta-analysis for osteonecrosis in patients 1-10 years of age

Single nucleotide polymorphisms (SNPs) (N=9,157) with associations with osteonecrosis ($P<.05$) in both the discovery and replication cohorts are shown in red or orange. The top coding SNP (rs34144324 in GRID2, meta-analysis $P=7.07x10^{-7}$) and loci reaching genome-wide significance ($PROX1-AS1$, rs1891059, $P=8.72x10^{-8}$; $LINC00251$, rs141059755, $P=1.30x10^{-8}$; $BMP7$, rs79085477 and rs75161997, $P=8.29x10^{-9}$) are noted.
Figure 1

Eligible patients enrolled on COG AALL0331 (N = 5,305)

Excluded (N=1312)
- Not randomized post-induction (N=1,094)
- Very high risk ALL genotype (N=165)
- Failed induction (N=29)
- Data unavailable (N=24)

Evaluable post induction therapy (N=3,993)

Patients (cases) with symptomatic osteonecrosis (N=111)

Excluded (N=3)
- Germline Trisomy 21 (N=3)

Patients with symptomatic osteonecrosis attempted genotyping (N=108)

Excluded (N=26)
- DNA/database sex differ (N=2)
- Genotyping Failed (N=10)
- After amendment 2C (N=14)

Analyzed (N=82)

Patients (controls) without symptomatic osteonecrosis (N=3,882)

Excluded (N=3,547)
- Sample not tested (N=3,501)
- Germline Trisomy 21 (N=6)
- <1000 days follow-up (N=40)

Patients without symptomatic osteonecrosis attempted genotyping (N=335)

Excluded (N=48)
- DNA/database sex differ (N=1)
- Genotyping Failed (N=27)
- After amendment 2C (N=20)

Analyzed (N=287)
Figure 2.
Figure 3.
Genetic risk factors for the development of osteonecrosis in children under age 10 treated for acute lymphoblastic leukemia