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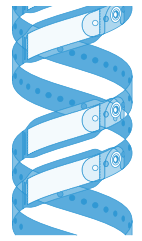
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A *GRIN3A* polymorphism may be associated with glucocorticoid-induced symptomatic osteonecrosis in children with acute lymphoblastic leukemia

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Aim: To evaluate the association between candidate genetic polymorphisms and glucocorticoid-induced osteonecrosis in Arab children treated for acute lymphoblastic leukemia. **Methods:** A total of 189 children treated for acute lymphoblastic leukemia were genotyped for four SNPs with allele discrimination assays. The incidence and timing of radiologically confirmed symptomatic grade 4 osteonecrosis were classified based on the Ponte di Legno toxicity working group consensus definition. **Results:** Thirteen children developed grade 4 osteonecrosis (6.8%), of whom 12 received the intermediate/high-risk treatment protocol. *GRIN3A* variant allele carriers had to stop dexamethasone therapy earlier resulting in significantly shorter duration of dexamethasone treatment (mean [95% CI]: 75.17 [64.28–86.06] vs 85.90 [81.22–90.58] weeks; $p = 0.054$) and lower cumulative dose (mean [95% CI]: 1118.11 [954.94–1281.29] vs 1341.14 [1264.17–1418.11] mg/m²; $p = 0.011$). **Conclusion:** This is the first pharmacogenomics evaluation of the association between *GRIN3A* variants and glucocorticoid-induced osteonecrosis in Arab children.

Lay abstract: This study aimed at uncovering variants in the genetic material of Arab children, that might predispose them to develop a specific treatment-related adverse effect, during their therapy for acute lymphoblastic leukemia (a type of blood cancer). We looked at specific changes in the DNA of our patient cohort that might predispose them to develop treatment-induced osteonecrosis. Osteonecrosis is by definition a loss of blood flow to the bone tissue in one's body, causing the bone to die. Osteonecrosis may be caused by long-term exposure to steroid-based medication, among which dexamethasone. Dexamethasone a main component of the combination of chemotherapeutic agents used to treat acute lymphoblastic leukemia. Our findings suggested that children who had one of the variants detected in a specific location of DNA, the *GRIN3A* gene, were more likely to develop osteonecrosis earlier and had to stop dexamethasone earlier during therapy.

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Keywords: acute lymphoblastic leukemia • Arab • children • dexamethasone • genetic polymorphisms • glucocorticoids • *GRIN3A* • osteonecrosis • pharmacogenetics

Acute lymphoblastic leukemia (ALL) in children is the most common cancer in childhood accounting for almost 30% of pediatric cancer cases in Lebanon, but with favorable cure rates reaching up to 80% in our institution [1]. Treatment-related toxicity is serious and sometimes life-threatening and is one of the main reasons for interruption or discontinuation of treatment which may ultimately affect disease outcome [2,3]. Although doses for antileukemic agents are calculated based on body surface area according to standardized protocols, prediction of toxicity is still difficult because of wide interindividual variability. Accordingly, investigators worldwide have tried to explore

predictors of toxicity in order to improve quality of life and identify a certain subgroup of high-risk patients. The field of pharmacogenetics (PGx) has hence emerged as a promising tool for the investigation of some genetically predisposing factors for drug toxicity [1,2,4]. The focus has so far been on the commonly used drugs, with the most compelling PGx evidence in ALL being for 6-mercaptopurine therapy and risk of myelosuppression [5,6].

Current ALL treatment protocols include a multi-drug regimen, of which glucocorticoids such as dexamethasone (DEXA) are a significant component. Almost all childhood ALL protocols rely on DEXA during the prolonged maintenance phase. Most protocols, including the one used at our institution, uses prednisone only during induction. One clinically relevant toxicity associated with glucocorticoids is osteonecrosis (ON), a dose-limiting pathologic process that affects osteocytes and causes cellular death. ON is a progressive condition that results in the destruction of joints within months to a year ultimately leading to mechanical failure of the affected bone [7]. The incidence of ALL-related ON ranges between 1.6 and 17.6% depending on the treatment protocol, ON severity classification and population [8]. No data are yet available in Arabs except for Egyptian children who were shown to have a 5-year cumulative incidence of 11.96% upon treatment for ALL [9]. At our institution, the American University of Beirut Medical Center (AUBMC), we have been treating children who are mostly Arabs with the St. Jude TOTAL XV protocol, and some developed severe and debilitating ON despite no evident risk factors beyond age and protocol arm, hence the need to elucidate additional factors such as genetic polymorphisms.

To date, a number of investigators evaluated genetic polymorphisms in relation to glucocorticoid-induced ON in children with ALL [10–18]. Most notably, and relevant to our patients, are results coming from St. Jude Children's Research Hospital as the same treatment protocol is used in our institution. For instance in 2008, a candidate SNP approach was performed to include variants that are known PGx markers of 6-mercaptopurine and methotrexate therapy, and showed a single nucleotide polymorphism (SNP) in *SERPINE1* to be associated with glucocorticoid-induced ON in children with ALL who were less than 10 years of age [13]. Nevertheless, patients at the time were receiving an older version of the St. Jude protocol (XIII and XIV) and the results may not be very clinically relevant as ON is more common in children who are 10 years or older. After that in 2011, a genome wide association (GWAS) analysis was performed on 364 children with ALL who received the St. Jude protocol Total XV, and revealed most significant results with a variant in *ACPI* (*rs12714403*) and another in *SH3YL1* (*rs4241316*) [16]. More recently in 2015, a much larger GWAS that entailed 2285 patients with two validation cohorts, one from the COG and another from St. Jude, revealed highly significant associations with two relatively common variants in *GRIN3A* (*rs10989692*) and *GRIK1* (*rs2154490*) [11].

The aim of this study was to determine whether previously identified genetic predictors, mainly *ACPI* (*rs12714403*), *SH3YL1* (*rs4241316*), *GRIN3A* (*rs10989692*) and *GRIK1* (*rs2154490*), of ON in children receiving DEXA as part of their combination chemotherapy regimen for treatment of ALL are also applicable to Arab children treated at our institution.

Methods

Human participants

This PGx study builds on two cohorts of previously recruited children who were treated for ALL at the Children's Cancer Center of Lebanon (CCCL) of the AUBMC. The CCCL is an NGO affiliated with the St. Jude Children's Research Hospital. The center aims to secure funds to help all cancer children in Lebanon (age ≤ 18 years) have access to the best and most up to date and evidence based management at zero cost. The center treats children coming from all cities of Lebanon. It also accepts patients coming from neighboring Arab countries such as Iraq and Syria.

The first cohort has been previously very well described [19,20]. It entails 143 patients recruited at the CCCL between 2010 and 2013, of whom 131 were included in this study as they were exclusively treated with the St. Jude protocol, they finished treatment and DNA was available for genotyping. The second cohort entailed clinical data and DNA from subjects who were recruited on a tumor banking biologic registry. Since 2015, this registry has been open for recruitment and clinical samples collection for all consenting patients treated for any cancer at the CCCL. Of the latter, 58 participants were included in this study as they were also treated exclusively with the St. Jude protocol and they finished treatment. Both research protocols were approved by our Institutional Review Board with the following numbers: PED.SM.05 and PED.RS.03, respectively. For both studies, parents and subjects – as applicable – have agreed and signed during the informed consent process that peripheral blood is withdrawn for DNA analysis and that their clinical records are reviewed. The current study was also approved

by the IRB: BIO-2019-0122 for retrospective collection of DEXA-related data from consented participants who finished treatment with the St. Jude protocol.

Treatment protocol

The treatment protocol at the CCCL was adopted from the St. Jude Total XV therapy with minor modifications. Briefly, it includes an induction phase with several drugs (glucocorticoids, vincristine, an anthracycline, asparaginase, cyclophosphamide, cytarabine and 6-mercaptopurine) to achieve clinical remission, followed by a consolidation phase (methotrexate and 6-mercaptopurine), and intrathecal therapy. Finally, a continuation phase that lasts 2 to 3 years from the time of diagnosis and involves weekly cycles of various combinations of drugs (mainly vincristine, asparaginase, 6-mercaptopurine, methotrexate, cyclophosphamide, cytarabine and DEXA) follows.

DEXA is given orally during the continuation phase of treatment according to the patients' disease risk stratification (high/standard risk and low risk). The continuation phase consists of 120 weeks for females and 146 weeks for males. A more intensive therapy termed 're-induction' is given for both risk groups. Re-induction Phases I and II last for 3 weeks each and occur at weeks 7–9 and 17–19, respectively, during continuation therapy. Low-risk patients receive DEXA 8 mg/m²/day for 2 weeks during each re-induction phase, and once monthly DEXA pulses of 8 mg/m²/day for 5 days during continuation. Intermediate/high-risk patients receive once monthly DEXA pulses of higher doses (12 mg/m²/day) over 5 days, and during re-induction phases (weeks 7–9 and 17–19), they receive twice-monthly DEXA pulses at 8 mg/m² (during re-induction phases) or 12 mg/m² over 7 days.

Data collection

Retrospective medical chart review was performed for baseline demographics (sex, age at diagnosis and protocol arm) and incidence of ON. Of note that at the CCCL, patients are not systematically screened for ON unless symptomatic. As such, magnetic resonance imaging (MRI) is done only for symptomatic patients with severe pain affecting their daily living. Therefore, only incidences of radiologically confirmed and symptomatic grade 4 ON, based on the Ponte di Legno toxicity working group consensus definition of ON associated with glucocorticoids in childhood lymphoblastic leukemia treatment, are available for analysis. ON is defined as: 'symptomatic' with deformation by imaging of one or more joints and/or substantially limiting self-care activity of daily living [21]. The cumulative dose of DEXA (in mg/m²) and time (in weeks) at which grade 4 ON occurred as evidenced by MRI, were recorded. For those who did not develop glucocorticoid-induced ON, these data were calculated until the last administered DEXA dose. It is notable that upon documentation of grade 4 ON, DEXA is withheld and replaced by methotrexate 40 mg/m².

Genotyping

DNA was isolated from peripheral blood using a DNA isolation kit from Qiagen (MD, USA) and stored at -20°C until genotyping. The four candidate genetic polymorphisms were measured by real-time PCR using Taqman[®] allele discrimination assays from Thermo Fisher Scientific (MA, USA) on the CFX instrument from Biorad (CA, USA) as per manufacturer's protocol, and as described in the authors' previous publications for other SNPs [19,20]. Genotyping for few samples was repeated for reproducibility.

Data analysis

Data were entered and analyzed using SPSS version 24.0 (IBM, USA). Continuous data are described as mean ± standard deviation (SD), while categorical data are reported as numbers and percentages.

Genotype frequencies were computed and tested for Hardy Weinberg Equilibrium (HWE) using a Chi-square test. Then, the association between baseline demographics (body mass index-BMI, age, sex and protocol arm), DEXA cumulative dose and genotypes with incidence of grade 4 ON were analyzed using unpaired Student's *t*-test or Fisher's exact test as appropriate. Univariate logistic regression was also performed to evaluate the odds ratio (OR) with 95% CIs of developing glucocorticoid-induced ON with the different genotypes. The cumulative DEXA doses and the time since starting DEXA therapy at incidence of grade 4 ON (if applicable) were also analyzed using Kaplan-Meier survival analysis with log-rank test with genotype as a covariate. Similarly, Hazard Ratios (HR) were computed with cox regression. Data were analyzed in the full cohort and within the intermediate to high-risk protocol arm only. No adjustment was performed for age as it is directly related to the protocol treatment arm with subjects allocated to the intermediate to high risk protocol arm being typically older. Due to the relatively small sample size, a recessive genetic model was also analyzed (i.e., wild type homozygous vs variant allele carriers).

Table 1. Frequency distribution of the evaluated single nucleotide polymorphisms (N [%]).

Gene	SNV	Homozygous wild type		Heterozygous		Homozygous variant allele		HWE p-value [†]	Sample MAF	Global MAF [‡]
<i>ACP1</i>	<i>rs12714403</i>	GG	172 (91.0)	GA	16 (8.5)	AA	1 (0.5)	0.359	0.05	0.09
<i>SH3YL1</i>	<i>rs4241316</i>	TT	173 (91.5)	TC	15 (7.9)	CC	1 (0.5)	0.295	0.04	0.09
<i>GRIK1</i> [§]	<i>rs2154490</i>	GG	124 (65.6)	GA	57 (30.2)	AA	8 (4.2)	0.657	0.19	0.23
<i>GRIN3A</i> [§]	<i>rs10989692</i>	GG	148 (78.3)	GA	36 (19.0)	AA	4 (2.1)	0.314	0.12	0.11

[†]p-values were computed by Chi-square test.
[‡]Global MAF (ALFA).
[§]Genotyping was not possible for one sample.
HWE: Hardy Weinberg equilibrium; MAF: Minor allele frequency.

A two-sided p-value of ≤ 0.05 is considered statistically significant.

Results

Sample characteristics & genetic polymorphisms

One hundred and eighty nine children were included in this study (105 males and 84 females) with a mean age \pm SD of 6.20 ± 4.57 years. The majority (81%) of the patients were Lebanese, and the rest were Arabs from different neighboring countries such as Syria, Palestine and Iraq. As shown in Table 1, and similarly to previous reports, *ACP1* and *SH3YL1* SNPs were uncommon and in linkage disequilibrium (LD) with a Pearson correlation of 0.972 ($p < 0.001$). The minor allele frequencies (MAF) of all four SNPs were similar to the global MAF, and all genotypes were in HWE ($p > 0.05$).

Thirteen children developed grade 4 ON and at multiple sites (6.8%). Nine were mainly affected at weight-bearing bones, two at nonweight-bearing bones and two at both. Of the 13 cases, 12 were enrolled in the intermediate- to high-risk treatment protocol. This protocol typically enrolls older children and entails higher DEXA doses, hence as expected, the affected patients were significantly older. Based on these findings, the bulk of the association analyses were done with the subgroup of patients who were treated with the intermediate- to high-risk protocol. Of note that, mean cumulative DEXA dose and treatment duration upon ON occurrence (if applicable) were significantly lower among those who developed ON (Table 2). As such, patients who developed severe clinical ON proved by MRI had to stop DEXA earlier during maintenance therapy and thus ended up receiving lower cumulative DEXA doses. Also, the duration of DEXA therapy was shorter.

Associations with glucocorticoid-induced ON

As shown in Table 1 & Supplementary Table 1, there were no significant associations between the different genotypes and glucocorticoid-induced ON even after adjustment for DEXA cumulative dose (Supplementary Table 2). Nevertheless, and within the sub-cohort of patients who received the intermediate- to high-risk treatment protocol only, *GRIN3A* variant allele carriers had significantly shorter duration of DEXA treatment (Mean time in weeks [95% CI]: 75.17 [64.28–86.06] vs 85.90 [81.22–90.58]; $p = 0.054$) (Supplementary Table 3 & Figure 1A) and lower DEXA cumulative dose (mean cumulative dose in mg/m^2 [95% CI]: 1118.11 [954.94–1281.29] vs 1341.14 [1264.17–1418.11], $p = 0.011$) (Supplementary Table 4 & Figure 1B). Of note that significant results were also revealed with the three different genotypes of *GRIN3A* and DEXA cumulative dose (Supplementary Table 4 & Supplementary Figure 1). The cox regression analysis which uses a different model of survival than the log-rank test did not reveal any significant results; nevertheless an almost significant trend appeared with the *GRIN3A* polymorphisms and DEXA cumulative dose: HR (95% CI): 0.60 (0.35–1.02); $p = 0.061$ for allele carriers versus wild type (Supplementary Table 5).

Discussion

The development of treatment-related ON can have serious and debilitating effects on pediatric ALL patients, with regard to treatment compliance and quality of life of survivors. The identification of genetic risk factors that will help in delineating high-risk subgroups of children becomes of utmost importance [11]. This study is the first PGx evaluation of the association between the above-mentioned candidate genes and glucocorticoid-induced ON in Arab children treated for ALL. We have shown that *GRIN3A* (*rs10989692*) variant allele carriers of that SNP

Table 2. Associations of baseline characteristics and genetic polymorphisms with incidence of glucocorticoid-induced grade 4 osteonecrosis.

Osteonecrosis			Full cohort (N = 189)			Intermediate/high-risk arm only (N = 88)		
			NO	YES	p [†]	NO	YES	p [†]
BMI	Kg/m ²	Mean ± SD	17.38 ± 3.47	17.55 ± 6.31	0.928	18.00 ± 3.70	17.81 ± 6.51	0.188
Age	Years	Mean ± SD	5.91 ± 4.41	10.07 ± 5.05	0.001	7.75 ± 5.47	10.66 ± 4.79	0.086
	<10 years	N (%)	143 (81.3)	6 (46.2)		48 (41.7)	5 (60.2)	
	≥10 years	N (%)	33 (18.6)	7 (53.8)	0.007	28 (58.3)	7 (39.8)	0.208
Sex	Males	N (%)	97 (55.1)	8 (61.5)		42 (55.3)	8 (66.7)	
	Females	N (%)	79 (44.9)	5 (38.5)	0.776	34 (44.7)	4 (33.3)	0.542
Protocol arm	Low	N (%)	99 (56.6)	1 (7.7)		–	–	
	Intermediate/high	N (%)	76 (43.4)	12 (92.3)	0.001	–	–	–
DEXA treatment duration [‡]	Weeks	Mean ± SD	86.80 ± 15.27	55.46 ± 27.44	<0.001	88.61 ± 15.00	54.66 ± 28.50	<0.001
DEXA cumulative dose [‡]	mg/m ²	Mean ± SD	1157.63 ± 294.14	837.07 ± 368.90	<0.001	1373.60 ± 271.85	841.50 ± 384.94	<0.001
ACP1 (rs12714403)	GG	N (%)	160 (90.9)	12 (92.3)		73 (96.1)	11 (91.7)	
	GA	N (%)	15 (8.5)	1 (7.7)		3 (3.9)	1 (8.3)	
	AA	N (%)	1 (0.6)	0 (0)	1.000	0 (0)	0 (0)	0.450
SH3YL1 (rs4241316)	TT	N (%)	161 (91.5)	12 (92.3)		73 (96.1)	11 (91.7)	
	TC	N (%)	14 (8.0)	1 (7.7)		3 (3.9)	1 (8.3)	
	CC	N (%)	1 (0.6)	0 (0)	1.000	0 (0)	0 (0)	0.450
GRIK1 (rs2154490) [§]	GG	N (%)	116 (65.9)	8 (61.5)		52 (68.4)	8 (66.7)	
	GA	N (%)	52 (29.5)	5 (38.5)		21 (27.6)	4 (33.3)	
	AA	N (%)	8 (4.5)	0 (0)	0.749	3 (3.9)	0 (0)	0.834
GRIN3A (rs10989692) [§]	GG	N (%)	139 (79.4)	9 (69.2)		62 (82.7)	8 (66.7)	
	GA	N (%)	33 (18.9)	3 (23.1)		12 (16.0)	3 (25.0)	
	AA	N (%)	3 (1.7)	1 (7.7)	0.287	1 (1.3)	1 (8.3)	0.139

[†]p-values were computed by two-sided Fisher's exact test for categorical data and Student's unpaired t-test for continuous data.

[‡]Although glucocorticoid-induced osteonecrosis is expected to occur at higher cumulative doses, the significantly lower cumulative dose and treatment duration at osteonecrosis is due to the fact that patients who developed severe clinical ON proved by MRI had to stop DEXA earlier during maintenance therapy and thus ended up receiving lower cumulative DEXA doses. Also the duration of DEXA therapy was shorter.

[§]Genotyping was not possible for one sample.

DEXA: Dexamethasone; MRI: Magnetic resonance imaging; ON: Osteonecrosis.

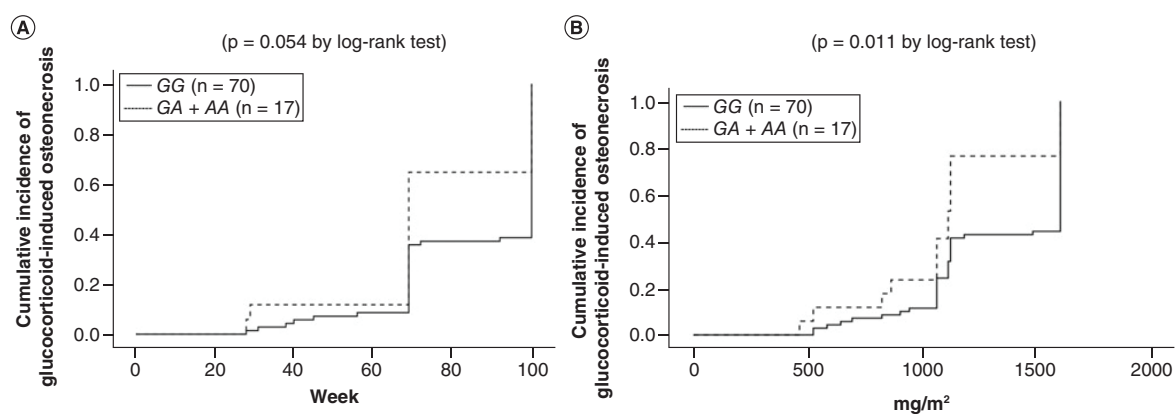


Figure 1. Cumulative incidence of glucocorticoid-induced grade 4 osteonecrosis in subjects treated with the intermediate-/high-risk arm by *GRIN3A* genotype (N = 88) with (A) duration of dexamethasone treatment and (B) cumulative dexamethasone dose.

are prone to earlier toxicity and at lower cumulative DEXA doses when compared with wild-type homozygous genotypes.

Glucocorticoid-induced ON

DEXA is a glucocorticoid that forms an essential component in the treatment of ALL. Studies have shown that glucocorticoids bind to their receptors and exert a cytotoxic effect through receptor dimerization and nuclear translocation, affecting gene expression. They can therefore inhibit the activity of certain transcription factors, such as AP-1 and NF- κ B and lead to cell cycle arrest and cytotoxicity [22–24]. Although the pathogenesis behind the occurrence of ON due to glucocorticoids remains unknown, some mechanisms have been advanced. For example, one study linked corticosteroid-induced ON with microemboli in the arterial vasculature supplying the bone. This is thought to occur after affecting circulating lipids [25]. Other studies claimed that corticosteroids cause changes in venous endothelium, induce blood stasis and elevate interosseous pressure, hence leading to ON [26]. A third proposed mechanism explains the occurrence of ON by hypertrophy and hyperplasia of adipocytes found in the bone marrow, impairing venous flow from osteocytes [27].

Previous studies have shown that glucocorticoid use is associated with ON with an incidence of 21–37% of patients on long-term glucocorticoid therapy, with the incidence varying widely according to the treated etiology and the treatment protocols [28]. The incidence of ALL-related ON ranges between 1.6 and 17.6% also depending on the treatment protocol, ON severity classification and population [8]. In the current study, the incidence of ON is 6.8% (13 out of 189) which is lower than that reported in previous studies that used the same treatment protocol for childhood ALL. As a matter of fact, Relling *et al.* [16] showed an incidence of 10.9% in their discovery COG AALL0232 cohort and 17.6% in their replication St. Jude Total XV cohort – both for grade 2–4 ON. Of note that the authors performed MRI as a routine surveillance and categorized ON based on the NCI Common Terminology Criteria for Adverse Events. They did not present data on the incidence of grade 3 and above ON based on the NCI criteria which is probably similar to grade 4 ON based on the criteria we used in this study [16]. More similarly to our findings, in a retrospective study done on Australian children treated according to the Australian and New Zealand Children's Hematology/Oncology Group, Padhye *et al.* [29] showed an ON incidence of 7.2% [29]. In this latter study, ON was defined as development of pain in bones or joints while on treatment or within 1 year of completion of treatment and confirmed by MRI. An incidence of ON as low as 1.2% was reported in a study done on a cohort of Italian children in Naples, treated on various ALL protocols though the definition of ON and/or grading was not specified in the study [30]. Interestingly, in a recent study done on Arab children of Egyptian origin with ALL, Ali *et al.* [9] showed an ON incidence of 9.7% during treatment according to St. Jude Total XV protocol. While this incidence reported is the closest to our study, it is important to note that ON detection was based on surveillance MRI and graded according to the Steinberg staging system of avascular necrosis [9]. To our knowledge, only the Nordic Society of Paediatric Haematology and Oncology group evaluated ON in children and adults treated for ALL using the Ponte di Legno toxicity working group consensus definition and found a 5-year cumulative incidence of 6.3% [31].

Candidate genetic polymorphisms & glucocorticoid-Induced ON

In this study, variant allele carriers of the *GRIN3A* (*rs10989692*) SNP were prone to earlier toxicity and at lower cumulative DEXA doses when compared with wild-type homozygous genotypes. *GRIN3A* is part of the glutamate NMDA ion channel superfamily and studies have highlighted its physiologic function in the central nervous system [32]. Other studies have also demonstrated a role for glutamate receptors in bone remodeling. For instance, it was shown that glutamate acts through ionic channels on osteoblasts and osteoclasts *in vitro* [33]. *In vivo*, the release of glutamate secondary to loading pressures has been demonstrated to increase bone mass [33]. *GRIN3A* is also thought to play an important role in the vascular system. In Taiwanese children with Kawasaki disease, a *GRIN3A* polymorphism was associated with a higher risk for coronary artery aneurysm formation [34]. As noted previously, Karol *et al.* [11] drew an association between *GRIN3A* and ON by relying on a large-scale GWAS. Furthermore, compelling evidence showed the *GRIN3A* variant to be involved in the various vascular phenotypes including cerebral ischemia, arterial embolism and thrombosis [11]. In addition, the disruption of the vascular supply to bone was shown to be a proximal event to glucocorticoid-induced ON in a murine model, data that drew further attention toward the *GRIN3A* variant [11].

In this study however, no significant associations appeared between *ACPI*, *SH3YL1* and *GRIK1* polymorphisms and ON. Polymorphisms in *ACPI*, a gene that plays a role in differentiation of osteoblasts, were shown to increase the risk for ON in a GWAS conducted on 364 children with ALL on the St. Jude protocol XV [16]. In the same study, another polymorphism in *SH3YL1* was also noted [16]. In 2015, Karol *et al.* [11] demonstrated an association between polymorphisms in *GRIN3A* and *GRIK1* and ON in 2285 children receiving dexamethasone for ALL

treatment on the Children's Oncology Group (COG) protocol. Those findings were also replicated in two cohorts of children with ALL on St. Jude protocol and children without ALL but treated with corticosteroids [11]. We believe that the lack of statistical significance with these candidate SNPs is partly due to the ethnic difference of our population compared with the studies by Kawedia *et al.* [16] and Karol *et al.* [11]. While most populations in these studies were of mixed races (Northern European, Hispanics, West African, East Asian), our study included Arab and Middle Eastern ethnicities only. In addition, our findings might be limited by the lower number of participants when compared with other GWAS.

Study limitations

This study suffers from some limitations. First, being retrospective in nature might hinder some of the data collection. Second, and as described above, we are missing ON of lower grades in our cohort due to the lack of screening protocol in our institution and imaging being done only after the onset of a severe pain crisis. Since this study builds on previously recruited subjects, no sample size calculation was performed. We however deliberately chose to genotype for the *GRIN3A* (*rs10989692*) and *GRIK1* (*rs2154490*) variants due to their relatively high minor allele frequencies when compared with the other two.

Conclusion

This study is the first to explore *ACP1* (*rs12714403*), *SH3YL1* (*rs4241316*), *GRIN3A* (*rs10989692*) and *GRIK1* (*rs2154490*) SNPs that might be linked to ON as a treatment related toxicity in a cohort of Arab children being treated for ALL. This investigation has the potential to help identify a specific subgroup of patients at high-risk for glucocorticoid-induced ON for personalized DEXA therapy.

Summary points

- The incidence of acute lymphoblastic leukemia (ALL) related osteonecrosis (ON) ranges between 1.6 and 17.6% depending on the treatment protocol, ON severity classification and population.
- No data are yet available in Arabs except for Egyptian children who were shown to have a 5-year cumulative incidence of ON of 11.96% upon treatment for ALL.
- At our institution, we have been treating children who are mostly Arab with the St. Jude protocol, and seeing some of them develop severe and debilitating ON despite no evident risk factors beyond age and protocol arm, hence the need to elucidate additional factors such as genetic polymorphisms.
- Our study is the first to explore *ACP1* (*rs12714403*), *SH3YL1* (*rs4241316*), *GRIN3A* (*rs10989692*) and *GRIK1* (*rs2154490*) SNPs that might be linked to ON as a treatment related toxicity in a cohort of Arab children being treated for ALL.
- Thirteen children developed grade 4 ON and at multiple sites (6.8%), of whom 12 were enrolled in the intermediate- to high-risk treatment protocol.
- Variant allele carriers of the *GRIN3A* (*rs10989692*) SNP were prone to earlier toxicity and at lower cumulative DEXA doses when compared with wild-type homozygous genotypes. *GRIN3A* carriers also had a lower DEXA cumulative dose.
- No significant associations appeared between *ACP1*, *SH3YL1* and *GRIK1* polymorphisms and ON.
- Some limitations of the study include its retrospective nature and the lack of screening protocols for ON which excluded patients with lower grades of ON from our cohort.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/pme-2020-0167

Author contributions

N Zgheib designed the research plan, completed the wet bench work with the help of her lab, analyzed datasets and wrote the manuscript. H El-Khoury, D Maamari designed the research plan, collected the data, contributed to the manuscript writing and provided meaningful discussion of main points. M Basbous designed the research plan and collected the data. R Saab designed the research plan, collected the data and revised the manuscript. S Muwakkit designed the research plan, collected the data and revised the manuscript. All authors have read and approved the final version of the manuscript.

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Ethical conduct of research

The study was approved by the Institutional Review Board: BIO-2019-0122. Parents and subjects – as applicable – have agreed and signed during the informed consent process that peripheral blood is withdrawn for DNA analysis and that their clinical records are reviewed.

Data sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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