Leukemia Research 38 (2014) 1055–1060

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Examination of *HFE* associations with childhood leukemia risk and extension to other iron regulatory genes

Amy E. Kennedy[a,1], Kala Y. Kamdar[b,2], Philip J. Lupo[b,3], M. Fatih Okcu[b,4], Michael E. Scheurer[b,5], Marianna K. Baum[c,6], M. Tevfik Dorak[a,*,7]

[a] *Department of Epidemiology, Robert Stempel College of Public Health & Social Work, Florida International University, 11200 SW 8th Street, AHC II 595-1, Miami, FL 33199, United States*
[b] *Department of Pediatrics, Section of Hematology-Oncology, Baylor College of Medicine, Houston, TX, United States*
[c] *Department of Dietetics and Nutrition, Robert Stempel College of Public Health & Social Work, Florida International University, Miami, FL, United States*

ARTICLE INFO

Article history:
Received 13 March 2014
Received in revised form 31 May 2014
Accepted 28 June 2014
Available online 11 July 2014

Keywords:
Genetic predisposition to disease
Childhood leukemia
Iron homeostasis
Iron regulatory gene markers

ABSTRACT

Hereditary hemochromatosis (*HFE*) variants correlating with body iron levels have shown associations with cancer risk, including childhood acute lymphoblastic leukemia (ALL). Using a multi-ethnic sample of cases and controls from Houston, TX, we examined two *HFE* variants (rs1800562 and rs1799945), one transferrin receptor gene (*TFRC*) variant (rs3817672) and three additional iron regulatory gene (IRG) variants (*SLC11A2* rs422982; *TMPRSS6* rs855791 and rs733655) for their associations with childhood ALL. Being positive for either of the *HFE* variants yielded a modestly elevated odds ratio (OR) for childhood ALL risk in males (1.40, 95% CI = 0.83–2.35), which increased to 2.96 (95% CI = 1.29–6.80) in the presence of a particular *TFRC* genotype for rs3817672 (*P*interaction = 0.04). The *TFRC* genotype also showed an ethnicity-specific association, with increased risk observed in non-Hispanic Whites (OR = 2.54, 95% CI = 1.05–6.12; *P*interaction with ethnicity = 0.02). The three additional IRG SNPs all showed individual risk associations with childhood ALL in males (OR = 1.52–2.60). A polygenic model based on the number of variant alleles in five IRG SNPs revealed a linear increase in risk among males with the increasing number of variants possessed (OR = 2.0 per incremental change, 95% CI = 1.29–3.12; *P* = 0.002). Our results replicated previous *HFE* risk associations with childhood ALL in a US population and demonstrated novel associations for IRG SNPs, thereby strengthening the hypothesis that iron excess mediated by genetic variants contributes to childhood ALL risk.

* Corresponding author.
E-mail addresses: akenn001@fiu.edu, kennedyae@mail.nih.gov (A.E. Kennedy), kykamdar@txch.org (K.Y. Kamdar), Philip.Lupo@bcm.edu (P.J. Lupo), mfokcu@txch.org (M.F. Okcu), scheurer@bcm.edu (M.E. Scheurer), baumm@fiu.edu (M.K. Baum), dorakm@hope.ac.uk, mtd3053@gmail.com (M.T. Dorak).
[1] Present address: National Institutes of Health, National Cancer Institute, Epidemiology and Genomics Research Program, Division of Cancer Control and Population Sciences, 9609 Medical Center Drive, Rm. 4E114, MSC 9763, Rockville, MD 20850, United States. Tel.: +1 240 276 6723; fax: +1 240 276 7921.
[2] Baylor College of Medicine, Department of Pediatrics, Section of Hematology-Oncology, One Baylor Plaza, MS-BCM305, Houston, TX 77030, United States. Tel.: +1 832 824 4163; fax: +1 832 825 4732.
[3] Baylor College of Medicine, Department of Pediatrics, Section of Hematology-Oncology, One Baylor Plaza, MS-BCM305, Houston, TX 77030, United States. Tel.: +1 713 798 2960; fax: +1 713 798 3658.
[4] Baylor College of Medicine, Department of Pediatrics, Section of Hematology-Oncology, One Baylor Plaza, MS-BCM320, Houston, TX 77030, United States. Tel.: +1 832 822 4242; fax: +1 832 825 4202.
[5] Baylor College of Medicine, Department of Pediatrics, Section of Hematology-Oncology, One Baylor Plaza, MS-BCM305, Houston, TX 77030, United States. Tel.: +1 713 798 5547; fax: +1 713 798 8711.
[6] Florida International University, Robert Stempel College of Public Health & Social Work, Department of Dietetics and Nutrition, 11200 SW 8th Street, AHC I 450, Miami, FL 33199, United States. Tel.: +1 305 348 2888.
[7] Present address: Liverpool Hope University, School of Health Sciences, HCA EW 208, Liverpool L16 9JD, United Kingdom. Tel.: +44 151 291 3236.

http://dx.doi.org/10.1016/j.leukres.2014.06.016
0145-2126/

1. Introduction

The hereditary hemochromatosis gene, *HFE*, has shown multiple associations with cancer susceptibility [1–7], including risk for childhood acute lymphoblastic leukemia (ALL) [8], which has been replicated [9]. In multiple cancers [1,2], including childhood ALL [9], the association of *HFE* variants with cancer risk gets stronger in interaction with a polymorphism in the transferrin receptor gene (*TFRC*). Since HFE and TFRC proteins biologically interact in iron transfer across membranes such as intestinal mucosa and placenta, the observed statistical interaction supports the notion that the involvement of *HFE* variants in cancer risk modification is mediated via their effect on body iron levels [10]. Body iron levels have long been known to be positively correlated with general cancer risk as several cohort studies have shown [11–14], and iron's carcinogenic effect has been well documented [15]. Thus, *HFE* associations with cancer have strong biological plausibility.

Recent genome-wide association studies (GWAS) have identified the *HFE* variant C282Y as a major determinant of body iron levels [10]. The mediation of iron homeostasis by genetic variants extends beyond the *HFE* gene, with the strongest association being *TMPRSS6* rs855791 [16]. We reasoned that if *HFE* associations are due to their effect on iron levels rather than linkage disequilibrium with nearby polymorphisms, other iron regulatory gene (IRG) polymorphisms should show similar associations with childhood ALL risk. To test our hypothesis that previously observed *HFE* associations are mediated via their effect on body iron levels, we aimed to expand our study beyond *HFE* variants to additional IRG polymorphisms, first by confirming previously shown *HFE* SNP associations with childhood ALL, and assessing novel SNPs in *TMPRSS6* and *SLC11A2* for their associations. To further test our hypothesis that *HFE* variants modify the risk for childhood ALL via their effects on iron levels, we also included the *TFRC* polymorphism that is known to interact with *HFE* variants in the genotyping scheme to test whether this interaction occurs. The additional IRGs do not interact with *TFRC* biologically, so we did not predict any other interaction. To test our hypothesis, we used a new case–control set, which was first validated by replicating known childhood ALL associations [17].

2. Subjects and methods

2.1. Subjects

Institutional Review Boards of Baylor College of Medicine (BCM) and Florida International University approved the study protocol. The case–control sample was from Houston, TX, consisting of 161 incident cases with childhood (<18 years at diagnosis) ALL diagnosed at Texas Children's Hospital from 2007 to 2012, and 231 healthy controls with the same age range (<18 years) contemporaneously and locally recruited. Thus, all subjects were less than 18 years of age, and exclusion criteria for both cases and controls were refusal to participate in the study and the diagnosis of any other cancer or disease. Representing the age peak typical of childhood ALL, 73% of cases were 1–5 years old. Subjects and their parents were approached to obtain informed consent for provision of epidemiological data with a questionnaire and a biological sample. The DNA samples were extracted from saliva or peripheral blood samples at BCM. The sample was multi-ethnic to allow us to examine effect modification of childhood ALL risk by ethnicity. Ethnicity was determined by the responses provided on the questionnaire by the children's parents. Our main interest was the contrast between non-Hispanic Whites (NHW) and Hispanic Whites (HW), since childhood ALL is very rare in African-Americans, and we had a very small number of African-Americans ($n = 17$) in the case group. Information on clinical subtype of ALL was collected from medical records, and 88% of the cases were diagnosed with early precursor B (early pre-B) ALL subtype.

2.2. SNP selection

We included two *HFE* variants known to influence body iron levels commonly known as C282Y (rs1800562) and H63D (rs1799945), as well as the *TFRC* variant S142G (rs3817672), which is known to interact with *HFE* variants in previously reported cancer associations [1,2,9]. As other IRG variants, we included the GWAS-identified iron-related SNP *TMPRSS6* rs855791 [16], as well as two additional SNPs we selected also from the *TMPRSS6* gene (rs733655) and the *SLC11A2* gene (rs422982) involved in the non-transferrin receptor-related iron transfer across membranes. The last two SNPs were selected as the promoter region haplotype tagging SNPs for these two genes. The selected SNP from *TMPRSS6* (rs733655) is 32 kb away and not in linkage disequilibrium with the GWAS-identified marker (rs855791) in the same gene according to the HapMap project European population data ($r^2 = 0.29$). Two more SNPs were included as ancestry-informative markers (AIMs) to adjust for the ethnic heterogeneity in the multi-ethnic sample to supplement the self-reported ethnicity data. The two SNPs were rs285 and rs2891, which were identified as AIMs in previous studies due to their largely different allele frequencies in major ancestral human populations [18,19]. Characteristics of each SNP are given in Table 1.

2.3. Genotyping

TaqMan allelic discrimination assay was the choice of method for genotyping. All SNPs were genotyped by TaqMan assays purchased from Life Technologies (Foster City, CA) on CFX96 real-time PCR instrument (Bio-Rad, Hercules, CA). The assay ID of each assay is given in Table 1.

2.4. Statistical analysis

Genetic associations (both crude and adjusted) were evaluated by logistic regression using Stata v.11 (StataCorp, College Station, TX). Two-way statistical interactions were also analyzed by logistic regression by including an interaction (product) term for the genetic variant and the potential effect modifier (age, gender or ethnicity) in the explanatory variables in multivariable analysis. All statistical tests were two-tailed and threshold for statistical significance was set at $P \leq 0.05$. All genetic associations, except the *TFRC* locus, were assessed by using the dominant genetic model which corresponds to variant allele positivity and coded as 1 for heterozygote and variant allele homozygote genotypes, and 0 for the common allele homozygosity (referent). Due to the low frequency of their variant alleles, the two *HFE* SNPs were pooled together by creating a new variable for the number of cumulative variant alleles at both SNPs (0 for no variant allele, 1 for variant allele at either SNP, 2 for heterozygosity at both SNPs (compound heterozygosity) or variant allele homozygosity at either SNP). To be consistent with the previous studies, *TFRC* SNP was analyzed in recessive model (by coding variant allele homozygosity as 1 and the other genotypes as 0). A similar approach was used for a polygenic risk model using the total number of variant alleles at two *HFE* variants and three variants in non-*HFE* (*TMPRSS6*, *SLC11A2*) IRGs (0 for no variant allele at any SNP, 1 for one or two variant alleles at any SNP, 2 for three or more variant alleles at any of the five SNPs). All statistical associations in the overall group were adjusted for the ethnicity variable which had four categories (NHW, HW, African-Americans, and others). The efficiency of statistical adjustment for ethnicity was double-checked by adjustment for each of the AIMs separately. Before proceeding to the statistical analysis of genetic associations, Hardy–Weinberg disequilibrium was ruled out in controls as a test for gross genotyping errors. Given the number of associations examined, we used a slightly more stringent statistical significance threshold of $P \leq 0.01$ in the interpretation of our results.

3. Results

3.1. HFE C282Y and H63D frequencies in the sample population

As expected, *HFE* C282Y mutation was more common in the NHW subjects: variant allele frequencies were 0.113, 0.030 and 0.011 in NHWs, HWs, and African-Americans, respectively. The H63D variant positivity also had some variation across ethnic groups with frequencies of 0.254, 0.151 and 0.032 in NHWs, HWs, and African-Americans, respectively. These variations were similar to those observed in HapMap project population samples. Only two cases and two controls (all NHWs) were compound heterozygotes for C282Y and H63D.

3.2. Univariable genetic marker analyses in the overall group

Genotype frequencies for each SNP were in Hardy–Weinberg equilibrium in the control group when analyzed for each ethnicity group. All associations reported below for the whole group were adjusted for self-reported ethnicity. Replacing the ethnicity variable by either AIM did not appreciably alter the results. As shown in Table 2, neither C282Y nor H63D showed an overall association with childhood ALL risk. The *TFRC* SNP, which was included in the study to assess its interaction with *HFE* SNPs did not show any individual association in the overall group. The three IRG variants all