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***FCGR2A* and *FCGR3A* polymorphisms in classical Hodgkin lymphoma by EBV status**

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We read with interest the study of Diamantopoulos et al. published in *Leukemia and Lymphoma* in which the authors analyzed the correlation between latent EBV infection and an *FCGR2A* polymorphism among 40 Greek-Caucasians with leukemic low-grade B-cell lymphoma, mainly chronic lymphocytic leukemia (N=23, 57.5%) and splenic marginal zone lymphoma (N=11, 27.5%) [1]. Patients were considered as EBV-positive based on the detection of the *BXLF-1* gene by PCR in a peripheral blood sample (viral load, 2164.2 copies per mL, range 79–15,600). The viral mRNA LMP1 and the EBV serological profile of the patients were also investigated. The correlation of the EBV expression with the germline *FCGR2A* single nucleotide polymorphism (SNP) rs1801274 found that the proportion of *FCGR2A* R patients in EBV⁺ patients (84.2%) was significantly higher than in EBV[−] patients (26.6%) ($p = 0.001$), with the following *FCGR2A* genotype distribution: 3 HH (19%), 10 HR (62%) and 3 RR (19%) for patients with a positive EBV load compared to 15 HH (71%), 4 HR (19%) and 2 RR (10%) for patients with a negative EBV load. The correlation between *FCGR2A* genotypes and LMP1 expression showed similar results [1].

The *FCGR2A* SNP rs1801274 is a non-synonymous SNP resulting in a histidine to arginine substitution at position 131, and FCγRIIA 131H variants have a higher affinity to human immunoglobulin (Ig) G1, G2 and G3 [2]. Diamantopoulos et al. hypothesized that NHL patients harboring FCγRIIA 131HH variants have better control of latency for EBV

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infection than patients with FC γ RIIA 131R [1]. They also hypothesized that *FCGR2A* SNP probably does not interfere with the EBV acquisition, as the majority of the patients were seropositive for EBV based on anti-VCA-IgG antibody positivity (N=36, 90%). Intrigued by the results of this study [1], we investigated the role of *FCGR2A* SNP as well as the *FCGR3A* SNP (rs396991) in a cohort of 239 newly diagnosed Classical Hodgkin Lymphoma (CHL) patients age 18 years and older prospectively enrolled from 2002 to 2009 in the University of Iowa/Mayo Clinic SPORE (Specialized Program of Research Excellence) Molecular Epidemiology Resource [3]. In this cohort, 202 patients were Caucasian, and the remainder were non-Caucasian (n=6) or unknown (n=31). All were negative for human immunodeficiency virus. A peripheral blood sample was collected from all patients at diagnosis and DNA for genotyping was extracted using standard protocols. The *FCGR2A* SNP was genotyped as part of a larger project using a custom Illumina Infinium array (Illumina, San Diego, CA) and the *FCGR3A* SNP was genotyped using a custom designed pyrosequencing assay. This study was approved by the Human Subjects Institutional Review Board at Mayo Clinic and the University of Iowa for the SPORE study and all patients provided written consent for participation. Clinical characteristic of the series and *FCGR2A* and *FCGR3A* genotyping are presented in Table I. The EBV status of the tumor was established by EBER *in situ* hybridization for 104/239 patients (48%) with available tissue, and was positive for 23 (22%) and negative for 81 (78%) patients.

Consistent with the observations of Diamantopoulos et al., we found a higher proportion of *FCGR2A* R patients in EBV⁺ CHL (N=20, 87%) compared to EBV⁻ CHL (N=55, 68%) ($p = 0.06$). The *FCGR2A* HH, HR and RR genotype distributions were 26 (32%), 37 (46%) and 18 (22%) in EBV⁺ CHL compared to 3 (13%), 11 (48%), 9 (39%) in EBV⁻ CHL ($p = 0.04$). To better decipher the specific correlation between *FCGR2A* and EBV-related CHL, we also analyzed as a “genetic control” the non-synonymous SNP rs396991 in *FCGR3A*, which is located on the same region of chromosome 1. We found that the *FCGR3A* genotype distribution was similar among EBV⁺ and EBV⁻ CHL (Table I), with a minor allelic frequency (MAF, *FCGR3A* V allele) of 41% in EBV⁺ and 37% in EBV⁻ CHL. Among 1521 controls included in SPORE, the *FCGR2A* HH, HR and RR genotype distribution was 387 (25%), 736 (48%) and 398 (26%), which is close to the distribution observed in EBV⁻ CHL. The *FCGR3A* genotype distribution in controls was *FCGR3A* VV (N=647, 11%), VF (N=644, 44%), FF (N=167, 44%) with a MAF of 34% which was similar to the CHL patients overall or by EBV status. We conclude that EBV⁺ CHL patients in our study had a higher frequency of the low affinity FC γ RIIA 131R which suggests an influence of the humoral response to EBV infection by FC γ RIIA.

CHL is one of the EBV-related lymphomas. The virus is present in Hodgkin and Reed-Sternberg cells in 20% to 50% of CHL cases in Western countries. The detection of EBV-encoded small RNAs (EBER) in malignant cells is considered to be the gold standard to determine the EBV status of the tumor [4]. EBV-related CHL typically expresses three viral proteins, EBV nuclear antigen 1 (EBNA-1), LMP1 and LMP2A. After an EBV infection, the number of infected B-cells decreases due to EBV-specific cytotoxic T-lymphocytes, and this immune response is thought to be influenced by host genetics (e.g., HLA class I region) [5]. Regarding the humoral response, the serologic profile after EBV infection is characterized

first by the occurrence of anti-VCA, anti-EA and anti-EBNA-2 followed by the appearance of anti-EBNA-1 and a decrease of EBNA-2 [6]. It has been well documented that a symptomatic EBV infection is a risk factor for EBV⁺ CHL, but not for EBV⁻ CHL [7]. Levin et al. recently reported that before EBV⁺ but not EBV⁻ HL diagnosis, an aberrant serologic profile is observed with a reduced anti-EBNA-1 to EBNA-2 ratio, which is characteristic of a defective response to latent EBV infection [8]. In the latter study, higher anti-EBV VCA IgG antibody titers were observed before the EBV⁺ HL diagnosis, which could be associated with an increase in EBV replication [8]. Interestingly, in HL patients with active disease, higher EBNA-1 antibody titers were correlated with lower plasma EBV-DNA concentration, suggesting functional activities of these antibodies, although not sufficient for complete control of EBV [9].

The *FCGR2A* SNP influences bacterial infection susceptibility and also autoimmune disease in relation to the different capacities of FCγRIIA allotypes to clear immune complexes [10]. For viral infections, one study showed that FCγRIIA 131RR HIV subjects progressed faster than FCγRIIA 131H carriers to a CD4⁺ cell count below 200/mm³, and this impact on CD4⁺ cell count was independent of viral load [11]. They also observed that HIV-1 immune complexes were more efficiently internalized by FCγRIIA 131HH compared to RR subject monocytes, probably due to the presence of IgG2 in the immune complex, which have a low affinity for FCγRIIA 131RR [11]. While, FCγR-mediated inhibition seems important during HIV infection [12], to our knowledge, no study reported a similar association between EBV and *FCGR2A* genotype.

We were also able to investigate whether the *FCGR2A* SNP influences CHL prognosis in the SPORE cohort. The median follow-up of whole cohort was 5 years (range, 0.04–9.75) and the 6-year event free survival (EFS) was 72.9% (95%CI, 64.8–79.7). EBV⁻ patients had a higher EFS rate compared to EBV⁺ CHL in the SPORE cohort (71.9% [58–89] vs 29.0% [7–100], $p = 0.007$), noting that the EBV⁺ group was based on only 23 cases and 11 events. Using an ordinal (per R allele) model, *FCGR2A* did not influence the EFS of the whole series (Hazard ratio [HR]=0.91; 95%CI, 0.64–1.31; $p = 0.63$), EBV⁻ CHL (HR=0.91; 95%CI, 0.48–1.74; $p = 0.77$) or EBV⁺ CHL (HR=0.77; 95%CI, 0.27–2.17; $p = 0.62$). No difference in EFS was observed when testing a dominant model for *FCGR2A* or for the *FCGR3A* SNP (data not shown). However, our study was limited by the low number of patients, especially in EBV⁺ group. Nevertheless, these first results suggest that the *FCGR2A* SNP is unlikely to have a role in the control of the disease.

These exploratory results need to be validated in a larger series of CHL with EBV tumor status, as well as directly correlate genotypes with plasma EBV DNA load, as was done by Diamantopoulos et al [1]. While, we did not have EBV load, a recent study showed the majority of EBER positive HL had a high EBV DNA load in plasma [9]. In addition, it was suggested by Diamantopoulos et al. that *FCGR2A* SNP probably does not affect the risk of acquisition of EBV infection [1]. It would be interesting in the context of HL to consider the correlation between *FCGR2A* SNP and a complete EBV antibody profile before the diagnosis of HL in epidemiologic cohorts with banked pre-disease specimens [8] and a global screening of humoral immune responses to EBV [13]. A recent study showed that patients with a persistent or reappearance of EBV in plasma after initiation of treatment had

an unfavorable outcome [14]. Whether the *FCGR2A* SNP impacts EBV load during the course of the treatment should also be considered.

In conclusion, we observed that a higher proportion of EBV⁺ CHL patients carried *FCGR2A* RR genotype compared to EBV⁻ CHL and unaffected controls, supporting a potential role of this SNP in the control of EBV-latent infection.

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References

1. Diamantopoulos PT, Kalotychoy V, Polonyfi K, et al. Correlation of Fc-gamma RIIA polymorphisms with latent Epstein-Barr virus infection and latent membrane protein 1 expression in patients with low grade B-cell lymphomas. *Leuk.Lymphoma*. 2013 Jan 23.
2. Bruhns P, Iannascoli B, England P, et al. Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. *Blood*. 2009; 113:3716–3725. [PubMed: 19018092]
3. Thompson CA, Maurer MJ, Cerhan JR, et al. Elevated serum free light chains are associated with inferior event free and overall survival in Hodgkin lymphoma. *Am.J.Hematol*. 2011; 86:998–1000. [PubMed: 22006790]
4. Kuppers R. The biology of Hodgkin's lymphoma. *Nat.Rev.Cancer*. 2009; 9:15–27. [PubMed: 19078975]
5. McAulay KA, Higgins CD, Macsween KF, et al. HLA class I polymorphisms are associated with development of infectious mononucleosis upon primary EBV infection. *J.Clin.Invest*. 2007; 117:3042–3048. [PubMed: 17909631]
6. Henle W, Henle G, Andersson J, et al. Antibody responses to Epstein-Barr virus-determined nuclear antigen (EBNA)-1 and EBNA-2 in acute and chronic Epstein-Barr virus infection. *Proc.Natl.Acad.Sci.U.S.A*. 1987; 84:570–574. [PubMed: 3025881]
7. Hjalgrim H, Askling J, Rostgaard K, et al. Characteristics of Hodgkin's lymphoma after infectious mononucleosis. *N.Engl.J.Med*. 2003; 349:1324–1332. [PubMed: 14523140]
8. Levin LI, Chang ET, Ambinder RF, et al. Atypical prediagnosis Epstein-Barr virus serology restricted to EBV-positive Hodgkin lymphoma. *Blood*. 2012; 120:3750–3755. [PubMed: 22972983]
9. Hohaus S, Santangelo R, Giachelia M, et al. The viral load of Epstein-Barr virus (EBV) DNA in peripheral blood predicts for biological and clinical characteristics in Hodgkin lymphoma. *Clin.Cancer Res*. 2011; 17:2885–2892. [PubMed: 21478335]
10. van Sorge NM, van der Pol WL, van de Winkel JG. FcgammaR polymorphisms: Implications for function, disease susceptibility and immunotherapy. *Tissue Antigens*. 2003; 61:189–202. [PubMed: 12694568]
11. Forthal DN, Landucci G, Bream J, et al. FcgammaRIIa genotype predicts progression of HIV infection. *J.Immunol*. 2007; 179:7916–7923. [PubMed: 18025239]
12. Forthal DN, Moog C. Fc receptor-mediated antiviral antibodies. *Curr.Opin.HIV.AIDS*. 2009; 4:388–393. [PubMed: 20048702]
13. Zheng D, Wan J, Cho YG, et al. Comparison of humoral immune responses to Epstein-Barr virus and Kaposi's sarcoma-associated herpesvirus using a viral proteome microarray. *J.Infect.Dis*. 2011; 204:1683–1691. [PubMed: 21990424]
14. Kanakry JA, Li H, Gellert LL, et al. Plasma Epstein-Barr virus DNA predicts outcome in advanced Hodgkin lymphoma: correlative analysis from a large North American co-operative group trial. *Blood*. 2013 Feb 5.

Table IClinical characteristics, *FCGR2A* and *FCGR3A* genotyping of classical Hodgkin lymphoma patients.

	All N=239 (%)	EBV+ N=23 (%)	EBV- N=81 (%)	<i>p</i> -value*
Median age (range)	38 (18–89)	27 (21–73)	38 (18–81)	0.26
Male	124 (52)	17 (74)	40 (49)	0.09
ECOG PS				
0–1	205 (86)	17 (74)	68 (84)	0.36
2–4	34 (14)	6 (26)	13 (16)	
Histological subtype				
Nodular sclerosis	161 (67)	14 (61)	62 (77)	0.06 [†]
Mixed cellularity	27 (11)	5 (22)	7 (9)	
Diffuse lymphocyte predominance	12 (5)	1 (4)	6 (7)	
Lymphocyte depletion	0 (0)	0 (0)	0 (0)	
Unclassified	39 (17)	3 (13)	6 (7)	
Ann Arbor Stage				
I–II	122 (52)	10 (45)	39 (49)	0.79
III–IV	113 (48)	12 (55)	41 (51)	
B symptoms	92 (39)	14 (61)	27 (33)	0.02
Hemoglobin level <10.5g/dl	32 (14)	3 (14)	11 (14)	0.97
ESR 50	50 (34)	6 (43)	18 (38)	0.73
White-cell count 15,000/mm ³	30 (13)	2 (10)	15 (19)	0.31
Lymphocyte count <600/mm ³	29 (13)	6 (29)	12 (15)	0.13
Albumin level <40g/l	103 (51)	9 (45)	34 (48)	0.82
<i>FCGR2A</i> (rs1801274)				
HH	56 (23)	3 (13)	26 (32)	0.04
HR	122 (51)	11 (48)	37 (46)	
RR	61 (26)	9 (39)	18 (22)	
R allele	183 (77)	20 (87)	55 (68)	0.06
<i>FCGR3A</i> (rs396991)				
VV	28 (12)	5 (22)	8 (10)	0.76
VF	114 (50)	9 (39)	44 (57)	
FF	85 (38)	9 (39)	25 (33)	
V allele	142 (62)	14 (61)	52 (67)	0.55

EBV, Epstein-Barr virus; ECOG, Eastern Cooperative Oncology Group; PS, performance status; ESR, erythrocyte sedimentation rate.

* *p*-value compares EBV⁺ and EBV[−] classical Hodgkin lymphoma.[†] *p*-value compares nodular sclerosis and mixed cellularity histology subtype.