

ORIGINAL ARTICLE

FcγRIIa-H131R variant is associated with inferior response in diffuse large B cell lymphoma: A meta-analysis of genetic risk

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Summary

Purpose: Low-affinity variants FcγRIIIa-V158F and FcγRIIa-H131R may alter response to rituximab-based chemotherapy in diffuse large B-cell lymphoma (DLBCL) but available clinical evidence is inconclusive. Our purpose was to explore their association in terms of treatment response.

Methods: We performed a meta-analysis of published literature to associate these variants with complete remission after upfront immunochemotherapy in DLBCL, and summarized the genetic risk using the model-free approach of generalized odds ratio (OR_G). PubMed and EMBASE search (up to July 2014) yielded five pertinent studies.

Results: FcγRIIa-H131R was associated with an inferior response to treatment (OR_G 0.67; 95%CI 0.46-0.97) and an additive mode of inheritance, with the genetic risk of

heterozygotes assigned in the middle between high affinity (H/H) and lower affinity (R/R) genotypes. This effect was unrelated to risk stratification, as no association was documented for FcγRIIa-H131R variant with the international prognostic index (IPI) (OR_G 1.02; 95%CI 0.79-1.31 for IPI 3-5 over 0-2). FcγRIIIa-V158F had no impact on treatment response but linkage disequilibrium and defective antibody-dependent cell-mediated cytotoxicity may have affected the outcome.

Conclusion: FcγRIIa-H131R but not FcγRIIIa-V158F may modify treatment response in DLBCL.

Key words: FcγRIIIa, FcγRIIa, lymphoma, meta-analysis, polymorphism, rituximab

Introduction

Functional polymorphisms of the receptors for immunoglobulins (Fc gamma Receptors, FcγR) have been implicated in human disease. Substitution of valine (V) to phenylalanine (F) at FcγRIIIa amino acid position 158 (FcγRIIIa-V158F; rs396991) or substitution of histidine (H) to arginine (R) at FcγRIIa amino acid position 131 (FcγRIIa-H131R; rs1801274) result in lower-affinity variants for IgG subclasses. Consequently, these variants may modulate response to rituximab-based immunochemotherapy for B-cell lymphomas through altering the interaction between anti-CD20 and the host.

Nonetheless, published evidence is still inconclusive [1]. We have performed a meta-analysis of pertinent studies in DLBCL to assess their impact on disease response after upfront treatment with rituximab-based regimens. We quantified the genetic effect using the model-free approach of OR_G [2] and explored the mode of inheritance using the degree of dominance index [3].

Methods

PubMed and EMBASE were searched up to July

2014 for pertinent studies on the topic, using “(fc gamma receptor* OR FCGR*) AND (polymorphism* OR variant)” as search terms. We manually scrutinized the reference lists of eligible articles for additional studies and complemented the search with the American Society of Hematology (ASH, 2004-2013) and European Hematology Association (EHA, 2006-2013) proceedings to identify potentially missing studies.

A study was considered eligible if all the following conditions were met: (a) It provided full genotype distribution for FcγRIIIa-V158F and/or FcγRIIIa-H131R; (b) had extractable data on DLCL patients; (c) used rituximab-based chemotherapy as upfront treatment. No language restrictions were imposed. Studies on mixed lymphoma histologies as well as second-line studies, salvage or post-transplantation treatment were *a priori* excluded. Studies solely in abstract form were not considered.

Two authors (PDZ, LSP) performed the search and any discrepancies were discussed and resolved by consensus. We sought for the following information: author, publication year, population size and origin, intervention, genotype distribution, IPI classification, response to treatment, and criteria used for response.

Treatment response was the main outcome of interest and was stratified as complete remission (CR), partial remission (PR) and stable disease (SD)/progressive disease (PD). The response was evaluated both as dichotomous outcome (CR over no-CR) and as graded (ordinal) outcome (with CR being the optimal outcome and SD/PD the worst). IPI classification [4] was the secondary outcome of interest, dichotomized as 3-5 over 0-2 grades. IPI determines both disease response and longitudinal outcomes after chemotherapy in DLBCL and was included to explore whether the genetic effect of FcγR variants on response is related or not to IPI risk.

The OR_G metric [5] was used to perform a single-step estimation of the genetic risk based on the mutational load. Previous methodologies require multiple model testing through genotype merging (recessive, dominant, additive), usually without *a priori* biologic justification, and inferences may be problematic when multiple genetic contrasts are significant. Moreover, these genetic contrasts are not independent to correspond to a χ^2 test. The OR_G metric provides a model-free, direct interpretation of gene-to-disease associations; it measures the odds of having the outcome of interest relative to the odds of not having the outcome of interest, when the affected subject has higher mutational load than the non-affected subject, i.e. the risk is proportional to the increased genetic exposure (with wild-type homozygous considered as having the lowest genetic exposure and homozygous mutants the highest) [2,6]. Relative effects were pooled across eligible studies to calculate the combined random-effect estimates (with 95% confidence intervals/95% CI). Heterogeneity was measured using the Cochran's Q test and I^2 [7]. Due to the low power of the Q test, a $p_Q < 0.10$ is suggestive

of significant heterogeneity. Hardy-Weinberg equilibrium (HWE) was considered as a quality criterion to rate individual studies, and was assessed using the χ^2 test [8]. Even if the OR_G is more suitable in the presence of deviation from HWE [6], we performed a complementary sensitivity analysis after exclusion of studies that deviated from HWE.

For significant effects, we further explored the mode of inheritance using the degree of dominance h index [9]. The degree of dominance assigns the genetic risk of heterozygotes between the two extreme homozygotes, using two independent genetic contrasts (additive and co-dominant). In brief, the additive model compares the effect of extreme homozygotes and the co-dominant model compares the effect of heterozygotes vs the average of two homozygotes. When the co-dominant model is significant, dominance is present regardless of the significance of the additive model. As a result, the mode of inheritance h will indicate dominance of the wild type allele for $-1 \leq h < 0$ or dominance of the mutant allele for $0 < h \leq 1$. If the additive model is significant and the co-dominant model is not, then perfect additivity is suggested and $h=0$ [9].

Results

The databases search (last updated July 7, 2014) retrieved a total of 1144 non-duplicate publications. After title and abstract screening, 1111 articles were excluded on the basis of relevance. The remaining 33 articles were evaluated in full text and their reference lists were screened for additional publications. A total of five articles met all the specified criteria [10-14] (the selection process is displayed in the flow chart (Figure 1)). The individual study characteristics and outcomes of interest are shown in Table 1. Four out of 5 studies referred to Caucasian populations and a single study was conducted in Asian population. All have used R-CHOP combinations, but the number of cycles and frequency of treatment administration differed. Four studies applied the same criteria for response [15]. One did not cite the criteria it used to evaluate response and genotype frequencies deviated from HWE ($p < 0.05$) [14].

There was no supporting evidence of association between FcγRIIIa-V158F and CR to immunochemotherapy (OR_G 0.85; 95%CI 0.52-1.38; $I^2=38\%$, $p_Q=0.16$ for homogeneity). After excluding the study [14] that deviated from HWE, the combined effects did not alter (OR_G 0.80; 95%CI 0.46-1.40; $I^2=48\%$, $p_Q=0.12$ for homogeneity). The association was also not significant for ordinal response (OR_G 0.95; 95%CI 0.50-1.82; $I^2=52\%$, $p_Q=0.10$ for homogeneity; Table 1). On the con-

Table 1. Summary characteristics of eligible studies and outcomes of interest

Author	Year	Population	N	Age (years)	Upfront treatment	Response	FcγRIIIa-V158F			FcγRIIIa-H131R			IPI	FcγRIIIa-V158F			FcγRIIIa-H131R				
							VV	FV	FF	HH	HR	RR		VV	FV	FF	HH	HR	RR		
Kim DH [10]	2006	Korean (Asian)	113	60	R-CHOP (median 6 cycles)	PD/SD	1	5	3	3	3	2	0-2	34	36	5	39	27	6		
						PR	5	6	0	4	6	1	3-5	18	18	1	20	13	3		
						CR	45	42	3	52	29	5									
							OR _G 0.40 (0.15-1.04)			OR _G 0.40 (0.16-0.98)			OR _G 0.82(0.40-1.71)			OR _G 0.96 (0.46-2.00)					
Mitrović Z [11]	2007	Croatian (Caucasian)	58	NR (18-76)	R-CHOP (minimum 3cycles)	PD/SD	4	5	1	4	4	2	0-2	9	18	5	15	13	4		
						PR	2	1	1	0	3	1	3-5	7	14	5	8	14	4		
						CR	10	26	8	19	20	5									
							OR _G 1.91 (0.62-5.85)			OR _G 0.54 (0.19-1.55)			OR _G 1.13 (0.45-2.81)			OR _G 1.68 (0.68-4.14)					
Fabiszewicz A [12]	2011	Polish (Caucasian)	87	57 (25-81)	R-CHOP (median 6 cycles)	PD/SD	1	4	0	1	4	0	0-2	12	26	28	20	30	16		
						PR	3	8	10	6	6	9	3-5	3	9	9	7	10	4		
						CR	11	23	27	20	30	11									
							OR _G 1.11 (0.52-2.38)			OR _G 0.61 (0.28-1.36)			OR _G 1.09 (0.48-2.48)			OR _G 0.83 (0.37-1.88)					
Ahlgimm M [13]	2011	German (Caucasian)	512 (263)*	68 (61-80)	*R-CHOP-14 (6 or 8 cycles CHOP+ 8R)	PD/SD + PR	10	26	12	19	23	6	0-2	92	152	55	128	128	43		
						CR	66	114	35	92	98	25	3-5	53	119	41	85	107	21		
							OR _G 0.62 (0.36-1.08)			OR _G 0.89 (0.52-1.55)			OR _G 1.20 (0.88-1.62)			OR _G 1.00 (0.73-1.35)					
							OR _G 1.28 (0.40-4.08)			OR _G 0.67 (0.46-0.97) [OR _G 0.56(0.35-0.90)]			OR _G 1.13 (0.87-1.46)			OR _G 1.02(0.79-1.31)					
Váróczy L [14]	2012	Hungary (Caucasian)	51	53 (18-86)	R-CHOP-14/21 (1-8 cycles)	PD/SD	4	2	2	n/r	n/r	n/r	0-2	n/r	n/r	n/r	n/r	n/r	n/r		
						PR	5	0	1	n/r	n/r	n/r	3-5	n/r	n/r	n/r	n/r	n/r	n/r		
						CR	20	10	7	n/r	n/r	n/r									
							OR _G 0.62 (0.36-1.08)			OR _G 0.89 (0.52-1.55)			OR _G 1.20 (0.88-1.62)			OR _G 1.00 (0.73-1.35)					
Combined effects							Model-free			OR _G 0.85(0.52-1.38) [OR _G 0.95 (0.50-1.82)]			OR _G 0.67 (0.46-0.97) [OR _G 0.56(0.35-0.90)]			OR _G 1.13 (0.87-1.46)			OR _G 1.02(0.79-1.31)		
							Additive			n/a			OR 0.50 (0.26-0.97)			n/a			n/a		
							Co-dominant			n/a			OR 0.92 (0.60-1.42)			n/a			n/a		
							h index			n/a			h=0			n/a			n/a		

PD: disease progression, SD: stable disease, PR: partial remission, CR: complete remission, IPI: international prognostic index, n/a=not applicable; *263 had received R-CHOP and were evaluable for response/ 512 were evaluable for IPI stratification; R: rituximab, CHOP: cyclophosphamide, vincristine, doxorubicin, prednisone, effects in brackets are for ordinal response

trary, FcγRIIIa-H131R was associated with an inferior response, estimated both as dichotomous (OR_G 0.67; 95%CI 0.46-0.97; I²=0%, p_Q=0.46 for homogeneity) and ordinal outcome (OR_G 0.56; 95%CI 0.35-0.90; I²=0%, p_Q=0.71 for homogeneity). These effects were consistent across studies (absence of statistical heterogeneity). The mode of inheritance indicated perfect additivity (h=0) for FcγRIIIa-H131R variant. The interpretation is that the genetic risk of heterozygotes would lie in the middle between the two homozygous, i.e the high affinity (H/H) and the lower affinity (R/R) genotype. There was no association between FcγRIIIa-V158F (OR_G 1.13; 0.87-1.46; I²=0, p_Q=0.83 for homogeneity) or FcγRIIIa-H131R variant (OR_G 1.02; 0.79-1.31; I²=0, p_Q=0.69 for homogeneity) and IPI index.

Discussion

DLBCL accounts for the majority of lymphoproliferative disorders [16] and IPI [4] remains the most valid tool to predict all longitudinal outcomes, including event-free, progression-free and overall survival [17]. In the present study, we reviewed the association of RIIa-H131R and FcγRIIIa-V158F variants with treatment response in DLBCL and found supportive evidence that RIIa-H131R variant is a significant moderator of treatment and results in lower CR rates. Notably, this effect appears independent of IPI risk stratification, given that RIIa-H131R variant was not associated with IPI score. On the contrary, no as-

sociation was found for the FcγRIIIa-V158F variant and treatment response.

The biologic activity of rituximab is mediated through three different mechanisms that include a direct pro-apoptotic effect, complement-mediated cytotoxicity and antibody-dependent cell-mediated cytotoxicity (ADCC) [18]. ADCC is considered a major contributor to anti-CD20 efficacy in B-cell lymphomas [19] and requires effector cells that bind the Fc portion of the antibody through the FcR. It has been shown that ADCC activity is potentiated for natural killer cells that express valine homozygous (V/V) FcγRIIIa-158 over phenylalanine homozygous (F/F) [20,21]. An analogous biologic effect is documented for FcγRIIIa-131 expressed in neutrophils and macrophages, where the substitution of arginine (R) to histidine (H) results in higher affinity and increased phagocytosis of IgG2-opsonized particles [22]. To date, these biologic alterations have not been associated either with better response rates or significant differences in longitudinal outcomes across individual studies in lymphomas [1], with some notable exceptions in follicular lymphoma [23].

Natural killer-mediated ADCC is impaired in DLBCL and FcγRIIIa expression is reduced over healthy controls, suggesting a reduced activation potential. How this phenomenon affects individual response to R-CHOP is yet to be determined [24] and may attenuate functional differences of FcRIIIa variants. Moreover, a non-significant association may also imply insufficient sample size

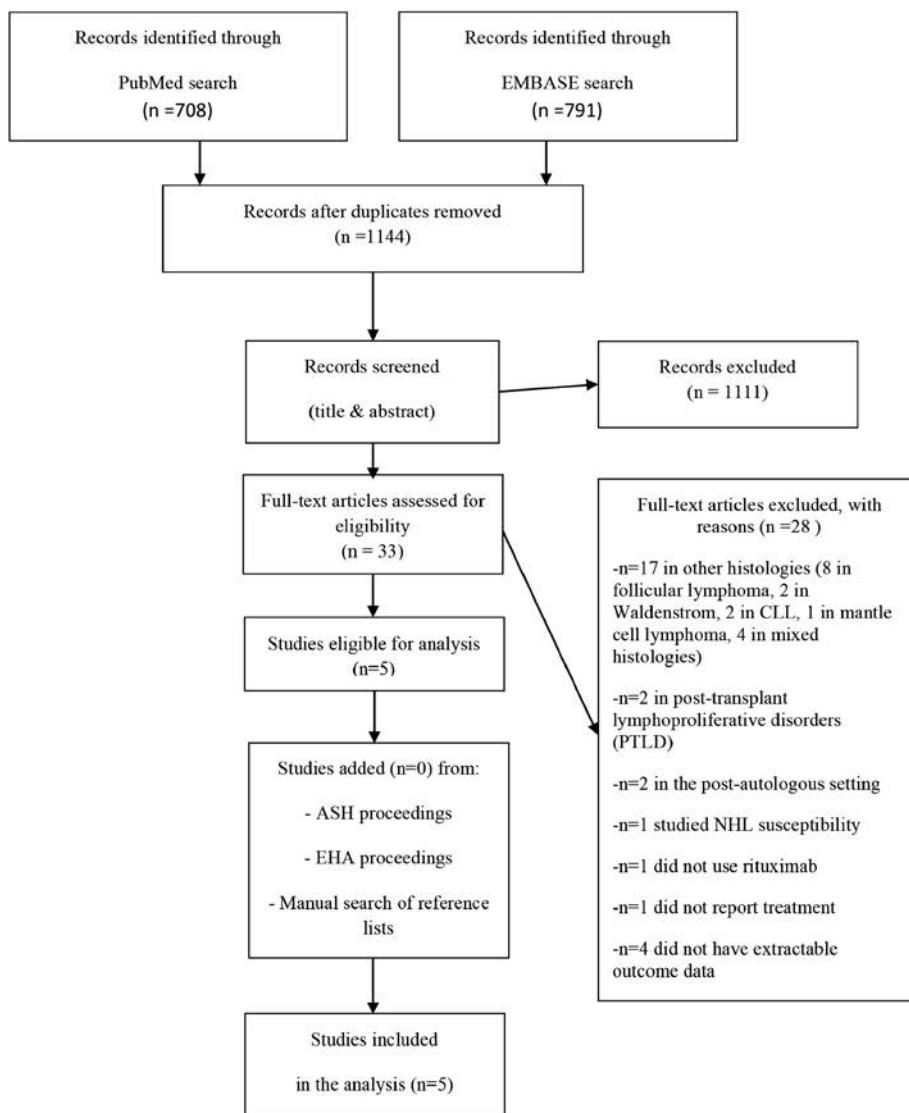


Figure 1. Flow chart showing the paper selection procedure.

rather than lack of biologic effect and meta-analysis may partially compensate for underpowered studies. However, population differences and clinical heterogeneity are inevitable and may moderate outcomes of interest; a meta-analysis cannot compensate for residual confounders. Furthermore, haplotype analysis and linkage disequilibrium could not be addressed due to the lack of bilocus genotype data across eligible studies. There is supporting evidence that FcγRIIa-H131R and FcγRIIa-V158F are in linkage disequilibrium among white patients [23,25] which may have impacted genetic effects.

In conclusion, this study provided supporting evidence that FcγRIIa-H131R but not FcγRIIa-V158F variant moderate disease response in DLBCL patients receiving rituximab as upfront treatment, and the genetic effect is unrelated to IPI risk stratification. In future studies treatment modifications may be warranted for low affinity

genotypes, particularly if suboptimal response to treatment is translated in different longitudinal outcomes.

Authors’ contributions

P.D. Ziakas conceived the idea, designed research, performed literature search, collected data, performed the statistical analysis and interpretation and wrote the manuscript. L.S. Poulou performed literature search, collected data, interpreted results and wrote the manuscript. E. Zintzaras designed the research, provided the analytical tools, performed the statistical analysis and interpretation and wrote the manuscript.

Conflict of interests

The authors declare no conflict of interests.

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