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FCGR3B Copy Number Loss rather than Gain is a Risk Factor for Systemic Lupus Erythematous and Lupus Nephritis: A Meta-analysis.

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Running title: *FCGR3B* Copy Number Loss is a risk factor for SLE and LN.

Key Words: Copy Number Variations, Systemic Lupus Erythematosus, Lupus Nephritis, Fc gamma receptor 3B.

Conflict of interest:
The authors have no conflicts of interest to declare.

Abstract:

Aim: A variety of studies have been performed to elucidate the association between Fc gamma receptor 3B (*FCGR3B*) copy number (CN) and the risk of systemic lupus erythematosus (SLE) and lupus nephritis (LN), yet results remain conflicting. Thus, we have undertaken a systematic review of all the studies published and carried out a meta-analysis to obtain a clearer understanding of the role of *FCGR3B* CN in the susceptibility of SLE and LN.

Method: A computerized literature search was conducted in databases of PubMed, ISI Web of Knowledge and systematically searched for all studies examining the association between *FCGR3B* CN and SLE or LN published up to May 2013.

Results: A total of 6 articles meeting all of the criteria above were included in this study. There were 5 comparisons of SLE including 2490 patients and 4286 controls and 4 comparisons of LN involving 689 patients and 1924 controls. Our results showed that individuals with *FCGR3B* CN gain could not increase risk of SLE or LN compared to normal genotype in the total analysis (SLE: OR = 1.00, 95% CI = 0.85-1.18, *P* = 0.97; LN: OR = 0.90, 95% CI = 0.69-1.18, *P* = 0.45). However, individuals with *FCGR3B* CN loss could increase risk of SLE and LN compared to normal genotype in the total analysis (SLE: OR = 1.77, 95% CI = 1.51-2.06, *P*<0.00001; LN: OR = 2.02, 95% CI = 1.59-2.57, *P*<0.00001).

Conclusion: In conclusion, our meta-analysis indicated that *FCGR3B* CN loss rather than CN gain was related to the susceptibility of SLE and LN.

Introduction:

Systemic lupus erythematous (SLE) is a prototypic, systemic autoimmune disease with a prevalence ranging from 15–50/100,000, characterized by pathogenic autoantibody overproduction, immune-complex deposition and multiple organ systems involvement [1]. Clinical manifestations are variable ranging from arthralgia, photosensitivity and the classic 'butterfly' rash to internal organ involvement, most notably renal and central nervous system disease [2]. Lupus nephritis (LN) is one of the most important clinical complications of SLE and also a major cause of morbidity and mortality in SLE. Susceptibility to SLE and/or LN is affected by a variety of genetic and environmental factors [3]. Copy number variation (CNV) of human DNA segments is an important source of genetic diversity and increasing evidence indicates that CNV may underlie disease susceptibility. The implications of CNV on phenotypic variation remain unclear, but specific copy number variants (CNVs) have been associated with susceptibility to numerous complex autoimmune diseases. Results from genome-wide linkage studies have suggested that the chromosomal region 1q23 is one of the strongest candidate regions for human SLE, in which the Fc gamma receptor (FCGR) gene cluster is found as an important locus [4-5]**.** The low-affinity FCGR gene family consists of four activating receptors (*FCGR2A, FCGR3A, FCGR3B* and *FCGR2C*) and one inhibitory receptor (*FCGR2B*) that have arisen through gene duplication and non-homologous recombination [6]. Although a number of single-nucleotide polymorphisms (SNPs) in FCGR genes have been associated with a variety of autoimmune disorders, the role of CNV in disease susceptibility has only recently begun to be investigated [6]. *FCGR2C, FCGR3A* and *FCGR3B* all exhibit CNVs [7] and, of these, a role for *FCGR3B* in risk of autoimmunity has been the most intensively investigated to date. The possible association between *FCGR3B* CNV and SLE and/or LN has been investigated with inconsistent results in various studies. Inconsistencies may arise from variation in sample size,or ethnic background. Thus, we have undertaken a systematic review of all the studies published and carried out a meta-analysis of the results to obtain a clearer understanding of the role of *FCGR3B* CN in the pathogenesis of SLE and LN.

Materials and methods:

Literature research

We considered all studies examining the association between *FCGR3B* CN and SLE and/or LN. A literature search for papers investigating CNVs of *FCGR3B* in SLE and LN was done on PubMed, ISI Web of Knowledge up to May 2013, using Medical Subject Heading (MeSH) terms and/or text words 'FCGR', 'Fc gamma receptor', '*FCGR3B* ', 'Fcg receptor 3B', 'copy number', 'copy number variation', 'copy number polymorphism', 'CN', 'CNV', 'CNP', 'Systemic lupus erythematous', 'SLE', 'lupus nephritis', 'LN'. Titles and/or abstracts were screened to estimate relevance of investigations. Full texts of primary selected literatures were downloaded for further study. Their references were hand-searched for potential related investigations. Articles were restricted to English language and analysis was restricted to data contained within a published peer-reviewed paper. When information about assay primers and the numbers of samples in each CN category (>2, 2 and<2) was not presented, the data were requested from the corresponding author.

Inclusion and exclusion criteria

A study was included in the meta-analysis if it was (i) published up to May 2013, (ii) case-control studies reporting the association of *FCGR3B* CNV and SLE and/or LN susceptibility, (iii) it was original data and discrete CN data were available. Major exclusion criteria as: (i) no sufficient data of CN distribution among the cohorts, (ii) reviews, (iii) duplication of previous studies.

Data extraction and statistical analysis

One author (Lijun Wu) searched for eligible investigations according to the inclusion and exclusion criteria listed above. Study characteristics including authors, countries, year of publication, ethnicity, sample size of cohorts, distribution of *FCGR3B* CN among the cohorts were extracted by 2 authors (Jin Yuan and Dongbao Zhao) independently. Data from the individual studies was then combined and an overall odds ratio for carrying 2 copies of *FCGR3B* relative to >2, or 2 relative to <2 were calculated using Mantel-Haenszel methods under a random effects model. Review Manager 5.0 software was used for all statistical analysis.

Results:

Characteristics of eligible studies

The search strategy and selection of articles were outlined in **Figure 1**. Sixteen records were searched through PubMed. Seven articles were excluded because of 3 reviews, 2 studies focused on other diseases, and 2 method studies, resulting in a total of 9 articles for detailed review. Three articles were excluded after individual abstract screening because of data not available and finally a total of 6 articles were included in this meta-analysis. SLE and LN were treated as two separate comparisons. There were 5 comparisons of SLE including 2490 patients and 4286 controls (**Table 1**) and 4 comparisons of LN involving 689 patients and 1924 controls (**Table 2**).

FCGR3B copy number variations and SLE susceptibility

In total, 5 comparisons investigating the *FCGR3B* copy number variations and its association with SLE susceptibility was identified. No significant association was found between high *FCGR3B* copy numbers (>2) and SLE susceptibility (**Figure 2.A**). Individuals with high *FCGR3B* copy numbers could not increase risk of SLE compared to normal genotype in overall analysis (OR = 1.00, 95% CI = 0.85-1.18, P = 0.97). However, there were significant associations between the low *FCGR3B* Copy numbers (<2) and SLE susceptibility (**Figure 2.B**). Individuals with low *FCGR3B* copy numbers could increase risk of SLE compared to normal genotype in the overall analysis (OR =1.77, 95% CI = 1.51-2.06, P<0.00001).

FCGR3B copy number variations and LN susceptibility

Four comparisons investigating the *FCGR3B* copy number variations and its association with LN susceptibility was identified. As results, similar findings were obtained as those in the SLE analysis. In detail, no significant association was detected between high *FCGR3B* copy numbers (>2) and LN susceptibility (**Figure 3.A**). Individuals with high *FCGR3B* copy numbers could not increase risk of LN compared to normal genotype in the total analysis (OR = 0.90, 95% CI = 0.69-1.18, P = 0.45). However, there were significant associations in the low *FCGR3B* copy numbers (<2) and LN susceptibility (**Figure 3.B**). Individuals with low *FCGR3B* copy numbers could

increase risk of LN compared to normal genotype in the overall analysis (OR = 2.02, 95% CI = 1.59-2.57, P<0.00001).

Discussion:

FCGR genes encode functionally diverse Fc gamma receptors, which recognize the Fc portion of immunoglobulin molecules. It is their specificity for different immunoglobulin isotopes and pattern of tissue expressions that define the FCGRs [9]. Low-affinity Fc receptors play a pivotal role in the initiation and regulation of the antibody-mediated immune response, linking humoral and cellular immunity [13]. These receptors recognize the constant domain of IgG and are involved in the mobilization of macrophages, natural killer T-cells and neutrophils to areas of immune complex deposition [13]. They also play an important role in the recognition and clearance of immune complexes, and as modulators of B-cell activity.

In our current meta-analysis, the *FCGR3B* copy number variations were associated with SLE susceptibility and the risk of LN. In overall analysis, low *FCGR3B* copy number could increase the risk of both SLE and LN, while there was no significant association between the high *FCGR3B* copy number and SLE or LN susceptibility. *FCGR3B* has a unique expression pattern largely restricted to neutrophils, where it is linked to the outer leaflet of the plasma membrane by a glycosylphosphatidylinositol anchor [14]**.** This function is known to be altered in SLE patients. Thus, low *FCGR3B* copy number may therefore result in reduced neutrophil trafficking to inflammatory lesions and an impaired ability to ingest immune complexes once there. A low copy number of *FCGR3B* was also initially found to be associated with the SLE sub-phenotype glomerulonephritis [15]. It is therefore plausible that reduced neutrophil expression of *FCGR3B* in patients with low *FCGR3B* copy number may lead to reduced glomerular clearance of immune complexes and susceptibility to autoimmune renal disease in patients with SLE.

However, some limitations still need to be further discussed. As with other meta-analysis, its interpretation must be bounded with the limited context. Primarily, further and larger-scales studies which could lessen the linkhood of type I and type II errors were needed. Thus, in some cases with small number of studies, the interpretation of

the results should be taken carefully. The limitations of current CN detection method are also partially responsible. A more accurate method of CN designation is therefore needed to confirm the association with SLE. In addition, studies published in English are more cited. Because of these limitations, caution should be exercised when interpreting the outcomes.

In conclusion, our meta-analysis confirmed that low *FCGR3B* copy number was related to the increased risk of both SLE and LN.

Acknowledgments

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Figure Legends:

Figure 1. Search results from PubMed and ISI Web of Knowledge and articles enrolled in our study.

Figure 2. Meta-analysis of studies investigating the *FCGR3B* copy number variations and SLE susceptibility. A: the comparison of high CN (CN gain) and normal CN; B: the comparison of low CN (CN loss) and normal CN.

Figure 3. Meta-analysis of studies investigating the *FCGR3B* copy number variations and LN susceptibility. A: the comparison of high CN (CN gain) and normal CN; B: the comparison of low CN (CN loss) and normal CN.

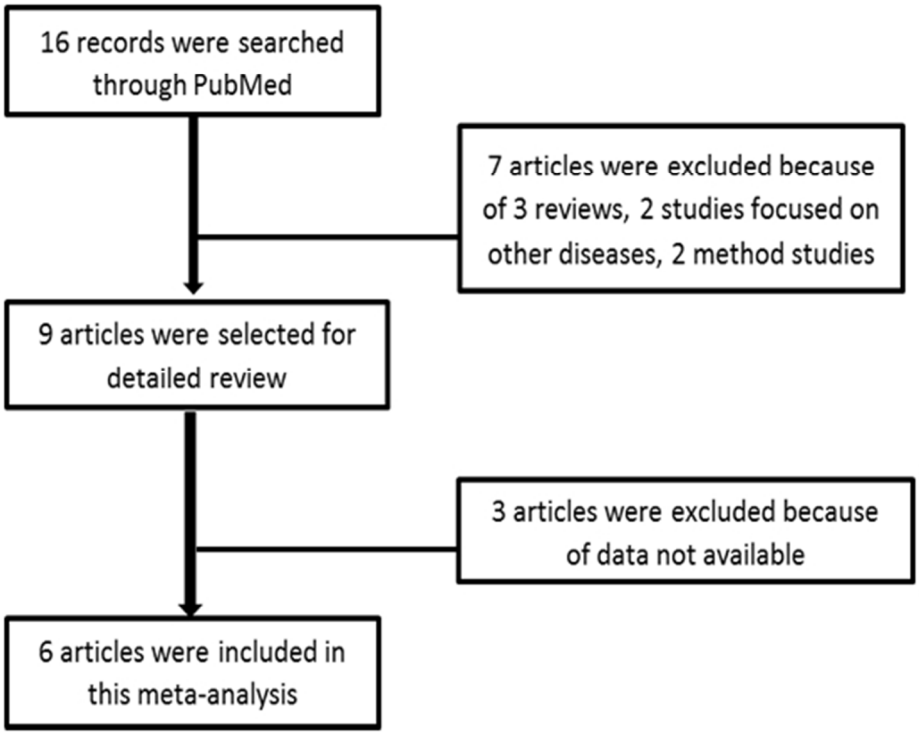

Figure 1. Search results from PubMed and ISI Web of Knowledge and articles enrolled in our study.
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Table 1 Characteristics of individual studies included in meta-analysis (Systemic lupus erythematous vs. Normal control)

Study	Year	Ethnic group	Detection method	Case	Control	CN<2		CN=2		CN>2	
						case	control	case	control	case	control
Molokhia[8]	2011	African	PRT assay	113	450	28 (0.248)	75 (0.167)	70 (0.619)	342 (0.760)	15 (0.113)	33 (0.073)
Morris[9]	2010	Caucasian	PRT assay	365	365	37 (0.101)	26 (0.071)	296 (0.811)	296 (0.811)	32 (0.088)	43 (0.118)
Niederer[10]	2010	Caucasian	PRT assay	450	1761	49 (0.109)	130 (0.074)	355 (0.789)	1434 (0.814)	46 (0.102)	197 (0.112)
		Chinese	PRT assay	880	989	134 (0.152)	97 (0.098)	627 (0.713)	754 (0.762)	119 (0.135)	138 (0.140)
Mamtani[11]	2010	Caucasian	Taqman	146	409	28 (0.192)	39 (0.095)	98 (0.671)	335 (0.819)	20 (0.137)	35 (0.086)
Fanciulli[4]	2007	Caucasian	SyberGreen	536	312	221 (0.412)	75 (0.240)	262 (0.489)	184 (0.590)	53 (0.099)	53 (0.170)

Table 2 Characteristics of individual studies included in meta-analysis (Lupus nephritis vs. Normal control)

Study	Year	Ethnic group	Detection method	Case	Control	CN <2		CN = 2		CN >2	
						case	control	case	control	case	control
Molokhia[8]	2011	African	PRT assay	31	450	10 (0.323)	75 (0.167)	19 (0.613)	342 (0.760)	2 (0.065)	33 (0.073)
Niederer[10]	2010	Chinese	PRT assay	348	989	62 (0.178)	97 (0.098)	243 (0.698)	754 (0.762)	43 (0.124)	138 (0.140)
Yang[12]	2010	Chinese	SybrGreen	149	173	20 (0.134)	19 (0.104)	49 (0.329)	68 (0.372)	80 (0.537)	86 (0.525)
Fanciulli[4]	2007	Caucasian	SyberGreen	161	312	73 (0.453)	75 (0.240)	81 (0.503)	184 (0.590)	7 (0.043)	53 (0.170)

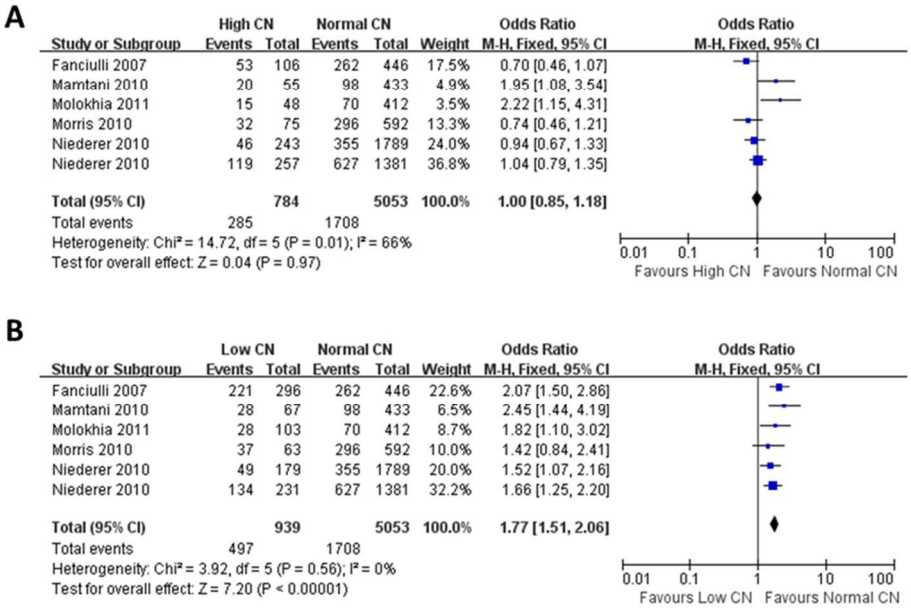

Figure 2. Meta-analysis of studies investigating the FCGR3B copy number variations and SLE susceptibility. A: the comparison of high CN (CN gain) and normal CN; B: the comparison of low CN (CN loss) and normal CN.
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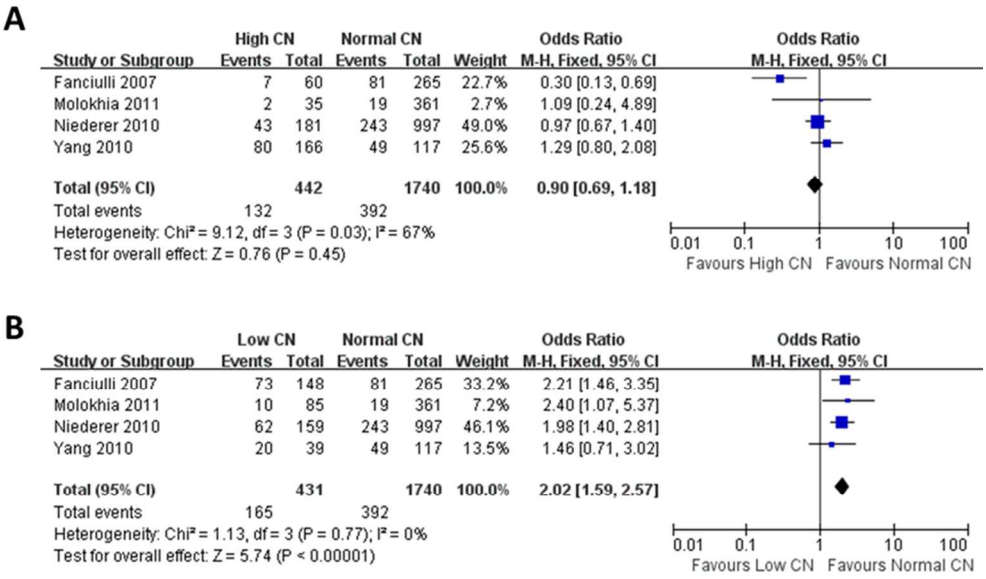

Figure 3. Meta-analysis of studies investigating the FCGR3B copy number variations and LN susceptibility. A: the comparison of high CN (CN gain) and normal CN; B: the comparison of low CN (CN loss) and normal CN.
80x50mm (300 x 300 DPI)