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Research Article

The Association of Human Leukocyte DQB1*02:01 Allele with Foetus and Neonatal Alloimmune Thrombocytopenia

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ABSTRACT

Background: Foetal and Neonatal Alloimmune Thrombocytopenia (FNAIT) is a condition resulted from the destruction of foetal Human Platelet Antigen (HPA-1a) by maternal antibodies. HLA-DQB1*02:01 allele is implicated in FNAIT but did not draw much attention. We conducted this study to investigate the correlation of HLA-DQB1*02:01 to FNAIT.

Study design and methods: We searched electronic databases to collect relevant studies (from inception to August 2021). Studies reported HLA-DQB1*02:01 genotype were included. HPA-1bb mothers who had confirmed FNAIT babies were called responders. HPA-1bb mothers who had been pregnant with HPA-1ab baby but did not develop FNAIT were called non-responders.

Results: Five eligible studies were included. Data were extracted to generate forest plots which show Odds Ratio (ORs), P-values, and 95% confidence intervals (ICs). Total number of responders and non-responders was 189 and 85 respectively. 143 of 189 responders (76%) were found to possess HLA-DQB1*02:01. In non-responders, only 29 of 85 (34%) were found to have HLA-DQB1*02:01. The odds ratios mean (95% C.I.) was statically significant (OR=6.60, P-value ≤ 0.001). This indicates that there is a strong association of HLA-DQB1*02:01 with responders. Therefore, we assume that HLA-DQB1*02:01 could be used as a complementary predictive risk factor with HLA-DRB3*01:01.

Conclusion: There is an obvious correlation of HLA-DQB1*02:01 with FNAIT. Future studies are needed to investigate the possibility of using HLA-DQB1*02:01 as a complementary risk predictor.

Keywords: Alloimmunisation; Thrombocytopenia; Human leukocyte antigens; Pregnancy

INTRODUCTION

Foetal and neonatal alloimmune thrombocytopenia

Platelets, also known as thrombocytes, play a crucial role in blood haemostasis and thromboses. Thrombocytopenia is a term refers to the reduction of platelet count [1,2]. Foetal and Neonatal Alloimmune Thrombocytopenia (FNAIT) is a condition caused by maternal alloantibodies against neonatal platelet antigens (HPAs) inherited from father, mostly HPA-1a [3-6]. These maternal alloantibodies (anti-HPA-1a) are Immunoglobulin G type (IgG) antibodies which can cross the placenta into the foetal blood circulation resulting in thrombocytopenia [7]. Placenta should prevent foetal blood from entering the blood circulation of the mother; However, foetal-maternal blood mix could occur when placenta detached after birth, miscarriage or fall incidence, or when prenatal test was carried out [3,7,8]. The majority of the severe thrombocytopenic foetuses and newborns were found to be complicated by FNAIT [9,10].

Characteristics of HPA-1a

In Caucasians, HPA-1a is the most frequent antigen that is implicated in severe FNAIT; HPA-1a induced FNAITs tend to be severe because antibodies against HPA-1a disrupt angiogenesis [6,11,12], the formation and development of new blood vessels. The antigenic system of HPA-1 is described as a leucine-to-proline amino acid substitution at position 33 of β3 integrin, also known as glycoprotein IIIa [11-13]. If leucine amino acid was on residue 33 (Leu33) replacing proline, then the product will be called HPA-1a, and HPA-1b if it was proline (Pro33). Heterozygous HPA-1ab is the inheritance of both amino acids, Leu33 and Pro33, while HPA-aa and HPA-bb is the homozygous status terminology. In order for FNAIT to occur, mother must lack HPA-1a, HPA-1bb mother; father must possess HPA-1a, either HPA-1ab or HPA-1aa father, to cause the baby to inherit HPA-1a, thus the baby

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must be HPA-1ab. There is a 50% chance for the foetus to inherit HPA-1a when the mother was HPA-1bb and the father was HPA-1aa; also, there is a 100% chance that the foetus will inherit HPA-1a if the mother was HPA-1bb and the father was HPA-1aa [14].

Clinical implications of FNAIT

The severity of the thrombocytopenia varies in FNAIT from asymptomatic, mild to severe thrombocytopenia [15,16]. Newborns might show signs and symptoms such as petechiae, haematomas, haematuria and blood in stools [15,16]. However, the most severe outcome of FNAIT is intracranial haemorrhage (ICH) which may lead to foetal death in utero or long-term disability to the child [16-20]. Furthermore, ICH occurs in approximately 10% to 30% of severe FNAIT cases, 20% of which is a chance of causing neurological sequelae to severe FNAIT cases, and a potential risk of death in 10% of ICH [11,19,21,22]. In addition, some studies state that 80% of ICH cases occurred in utero [11,23-26]. Although there are some strategies to manage the high rate of recurrence of severe thrombocytopenia in subsequent pregnancies, currently there is no reliable maternal screening to identify pregnancies at risk [27]. In this study we focus on the association of Human Leukocyte Antigens (HLAs) with FNAIT.

The association of human leukocyte antigens with FNAIT

Human Leukocyte Antigens (HLAs) are proteins on cell surfaces which encoded by genes of the Major Histocompatibility Complex (MHC) [28-30]. HLAs play a crucial role in mounting the immune response by presenting peptide fragments to specific T cells [29-31]. HLAs consist of two main classes, HLA class I (HLA-A, HLA-B, HLA-C) and HLA class II (HLA-DQ, DR, DP) [28-33]. Generally, foetal HPA-1a will be presented on the surface of Antigen Presenting Cells (APCs), by HLA class II, to be then recognised by T helper cells [34,35]. After a complicated immune response, B cells will respond to T cell signalling and differentiate to plasma cells to produce specific alloantibodies [36]. However, the inheritance of specific alleles of HLA class II plays a significant role in the occurrence of FNAIT. HLA-DRB3*01:01 is associated with the vast majority of FNAIT cases, up to 94% [3,8,11,29-37]. This indicates that HPA-1bb mothers, who possess HLA-DRB3*01:01, are at high risk of FNAIT while HPA-1bb mothers who lack this allele have low chance of developing FNAIT [38]. Some studies suggest that HLA-DRB3*01:01 has high avidity to bind and present HPA-1a peptides to generate T cell response [11]. Another allele, HLA DQB1*02:01, shows high percentages in FNAIT cases in all included studies in this systematic review [8,11,37-39].

FNAIT is one of the most serious conditions that might lead to the most severe outcome; ICH, to affected babies [16-18]. Therefore, genotyping HPA-1a negative mothers for HLA alleles has been a very useful approach to predict FNAIT occurrence to prevent it. HLA-DRB3*01:01 was the most useful allele to be used as a stratification risk factor for FNAIT due to its strong association with the majority of FNAIT cases. Despite the invaluable benefit of HLA-DRB3*01:01, some HPA-1bb mothers who developed FNAIT to their babies did not possess HLA DRB3*01:01. Therefore, it is crucial to investigate other HLA alleles to see if there will be an allele that could further predict FNAIT incidence. In addition, numerous studies and systematic reviews have broadly investigated the association of HLA-DRB3*01:01 with FNAIT. However, the obvious overrepresentation of HLA-DQB1*02:01 in confirmed FNAIT cases has not drawn much attention. Therefore, we hypothesised that investigating the association of HLA-DQB1*02:01 allele would be beneficial to further promote our knowledge of FNAIT complications, management, and treatment. Furthermore, expanding our understanding of FNAIT is crucial as there is no reliable screening program during pregnancy that can prevent FNAIT and HLA DQB1*02:01 allele might become a valuable predictive tool [27]. This study aims to 1) investigate the association of HLA-DQB1*02:01 allele with FNAIT; 2) determine the tendency of HPA-1bb women who possess HLA-DQB1*02:01 to undergo FNAIT; 3) promote the prediction of FNAIT using HLA-DQB1*02:01 allele.

METHODOLOGY

Study design

Present study was carried out according to the Preferred Reporting Items for Systematic Review and Meta-Analysis Statement (PRISMA) for evaluation of healthcare intervention [40].

Search sources and strategy

The review was conducted using different electronic databases, PubMed, Scopus, and ProQuest. Relevant articles published from inception to August 2021 were collected. Keywords and headings were entered in these databases solely, in combinations, as abbreviations or/and full forms. Key Boolean operators, "and", and "or" were used accordingly. The words were "foetal and neonatal alloimmune thrombocytopenia", "FNAIT", "NAIT", "HLA-DRB3 and FNAIT", "HLA DRB3*01:01", "HLA DQB1*02:01", "HLA class-II and neonatal thrombocytopenia", "HLA-DQB1", "neonatal thrombocytopenia".

Study screening and eligibility

Pre-adopted inclusion and exclusion criteria were set for study eligibility and screening. The inclusion criteria were: 1) English-written articles, 2) records that reported two study groups were included; responders and non-responders. Responders are immunised HPA-1bb women whose babies were confirmed with FNAIT due to anti-HPA-1a antibodies. Non-responders are non-immunised HPA-1bb women who had been pregnant for HPA-1ab foetus at least once but did not develop FNAIT. Exclusion criteria based on topic and abstract were: 1) duplicate records, 2) non-English, 3) clearly unrelated articles, and 4) animal model studies. Exclusion criteria based on full text: 1) Alloimmunisation of low frequency HPAs, 2) records that did not report HPA-DQB1*02:01 genotype, 3) records that focused on alloantibodies to HLA system rather than HPAs, and 4) studies that did not report non-immunised HPA-1bb women who have been pregnant with HPA-1ab foetus.

Data management and extraction

Microsoft Excel was used to calculate, and analysis extracted data. The statement of strengthening the Reporting of Observational Studies in Epidemiology (STROBE) was adopted to estimate the fitness of the included studies and to assess bias [41].

Statistical analysis

OpenMeta[Analyst]® program was used for undertaking meta-analysis and creating Forest Plots (this software is available from (http://www.cebm.brown.edu/openmeta/). OpenMeta [Analyst] was adjusted on diagnostic DerSimonian-Laird random-effect model to perform sensitivity and specificity of data. All results were represented in 95% confidence interval (CI). Odds ratio and P values were calculated automatically by OpenMeta[Analyst]® software. Odds ratio was calculated to show the strength of the association between some HLA alleles with FNAIT. Odds ratio>1 and P value ≤ 0.05 were considered statistically significant and, therefore, very associated.

RESULTS

Study selection

The entire process of the study selection is summarised in PRISMA flow chart (Figure 1). A total number of 855 records were identified through PubMed (n=221), Scopus (n=238) and ProQuest (n=396). 507 records remain after duplicates were removed by EndNote reference software. 436 articles were removed based on topic and abstracts, and reasons for exclusion were: 1) non-English, completely unrelated, and animal model studies. Full thirty-six studies were assessed for eligibility. After reading all content, 31 articles were excluded due to the following reasons: 1) twelve studies were excluded because their content which was about low frequent HPAs while we focus on HPA-1a as it is the most implicated antigen in FNAIT (3, 7, 8, 2) five studies were excluded because HLA-DQB1*02:01 genotyping was not reported, and 3) thirteen studies were about alloantibodies against HLA antigens. Seven studies were reviewed multiple times, yet two of which were considered ineligible; 1) one study has reported only one FNAIT case, 2) another study (3) has used general population as a control while our study focuses on non-responders as a control [34]. Therefore, five studies were included for the quantitative meta-analysis for this systematic review [8,11,37-39].

Study characteristics

A summary of the five included studies is shown in Table 1 [8,11,37-39]. The included studies have met the adopted criteria and were considered eligible. The interest of this systematic review is HLA-DQB1*02:01 allele; thus, all included studies provided data that we needed regarding this allele. The main focus of all five studies relates to different aspects of HLA-DRB*01:01 [8,11,37-39]. All included studies reported two female groups which serve the aims of this study. Responders which is the study population, and non-responders which is the control. Responders are HPA-1bb females who developed alloimmunisation and had foetuses or/and newborns with confirmed FNAIT. Non-responders are HPA-1bb females who had been pregnant with HPA-ab but did not develop alloimmunisation. The terminology of non-immunised and immunised HPA-1bb women is different in the included studies, but the main concept is the same. For example, some studies refer to HPA-1bb females as negative HPA-1a females and this is obviously another terminology. The study design of All five included studies were retrospective. All five studies reported the genotype HLA-DRB3*01:01 as well as other different HLA alleles. Most importantly, all five studies reported HLA-DQB1*02:01 genotype for responders and non-responders. In this systematic review, the control group of all five studies was non-responders because we needed to focus on females rather than whole populations, and thus many studies were excluded.

The total number of responders was 189 in all five studies; 36 in L'Abbe et al., 45 in Delbos et al., 23 in Loewenthanal et al., 71 in Sainio et al., and 14 in Sukati et al. [8,11,37-39]. The foetal HPA that was implicated in all studies was foetal HPA-1a. The number of responders who possessed HLA-DQB1*02:01 from each study was 34 (94%), 29 (64%), 17 (74%), 51 (78%), and 12 (85%) respectively. In comparison, the total number of non-responders was 85; 10 in L'Abbe et al., 19 in Delbos et al., 24 in Loewenthanal et al., 25 in Sainio et al., and 7 in Sukati et al. [8,11,37-39]. The number of non-responders who possessed HLA DQB1*02:01 allele from each study was 3 (30%), 6 (32%), 9 (37%), 10 (40%), and 1 (14%) respectively. The countries of the studies were Canada, France, Israel, Finland, and Scotland respectively.

Study fitness and quality assessment

The quality assessment of all five included studies according to STROBE checklist. Overall, fulfilment of STROBE was acceptable although it was not greatly fulfilled due to the following reasons, 1) sample size of all five studies was not predetermined, 2) there is a possible risk of selection bias towards the association of only HLA-DRB3*01:01 with confirmed FNAIT cases, 3) the description of the study population in Loewenthanal et al. 2013 study was inadequate [8,11,37-39]. However, the level of significance of sample size was fulfilled. All studies reported the genotype for both alleles; however, there was different tabulating in the result section in two studies [11,38] which led to insufficient information about HLA-DQB1*02:01 regarding the frequency of it in responders and non-responders who lack HLA-DRB3*01:01. All studies covered almost all aspects of HLA-DRB3*01:01, yet they did not provide enough justifications of the obvious overrepresentation of HLA-DQB1*02:01 in confirmed FNAIT cases (Table 2).

The overrepresentation of HLA-DQB1*02:01 in FNAIT

Table 3 summarises the odds ratios, 95% confidence interval (IC), and percentages of the association of HLA-DQB1*02:01 and HLA-DRB3*01:01 alleles in responders and non-responders. The mean of percentages and odds ratios as well as the total of responders and non-responders were provided in this table (Table 3). In responders, there is 76% (143/189) mothers have HLA-DQB1*02:01 allele compared to only 34% (29/85) in non-responders. HLA-DRB3*01:01 was high in responders as expected (93%) compared to only 26% of non-responders. This supports the known idea that HLA-DRB3*01:01 is strongly associated with confirmed FNAIT cases. However, HLA-DQB1*02:01 is also associated with responders because of the high percentages in all included five studies.

The forest plot in Figure 2 shows the odds ratio and P-value of the association of HLA-DQB1*02:01 with responders. The size of the squares in the forest plots indicates the number of responders and non-responders, the bigger the box, the greater number of participants in the study. The dashed line represents the odds ratio mean. The percentages of HLA-DQB*02:01 in responders were 94%, 64%, 74%, 78%, and 85% in L'Abbe et al., Delbos et al., Loewenthanal et al., Sainio et al., and Sukati et al. respectively [8,11,37-39]. The average was 76% compared to 34% in non-responders. The mean of all odds ratio was 6.60 and considered statistically significant, the lower bound was 2.906, and the upper bound was 14.987. P-value was ≤ 0.001 suggesting strong correlation of HLA-DQB1*02:01 in confirmed FNAIT cases (responders). The P-value of the heterogeneity was 0.144 indicating acceptable sample sizes of all five studies.

The long-known association of HLA-DRB3*01:01 allele with FNAIT

The forest plot generated for HLA-DRB3*01:01 allele. It demonstrates the inheritance of HLA DRB3*01:01 in responders and non-responders and its strong association with responders. The percentages of HLA-DRB3*01:01 in responders were 91%, 84%, 87%, 100% and 93% in L'abbe et al., Delbos et al., Loewenthanal et al., Sainio et al., and Sukati et al. respectively [8,11,37-39]. The average was 93% compared to 26% of non-responders. The mean of all odds ratio was 41.429 and the P-value was ≤ 0.001 confirming the strong association of this allele with FNAIT. The lower bound of the odds ratio was 17.323, and the upper bound was 99.081. The P-value of the heterogeneity was 0.700 indication acceptable sample size of all five studies (Figure 3).

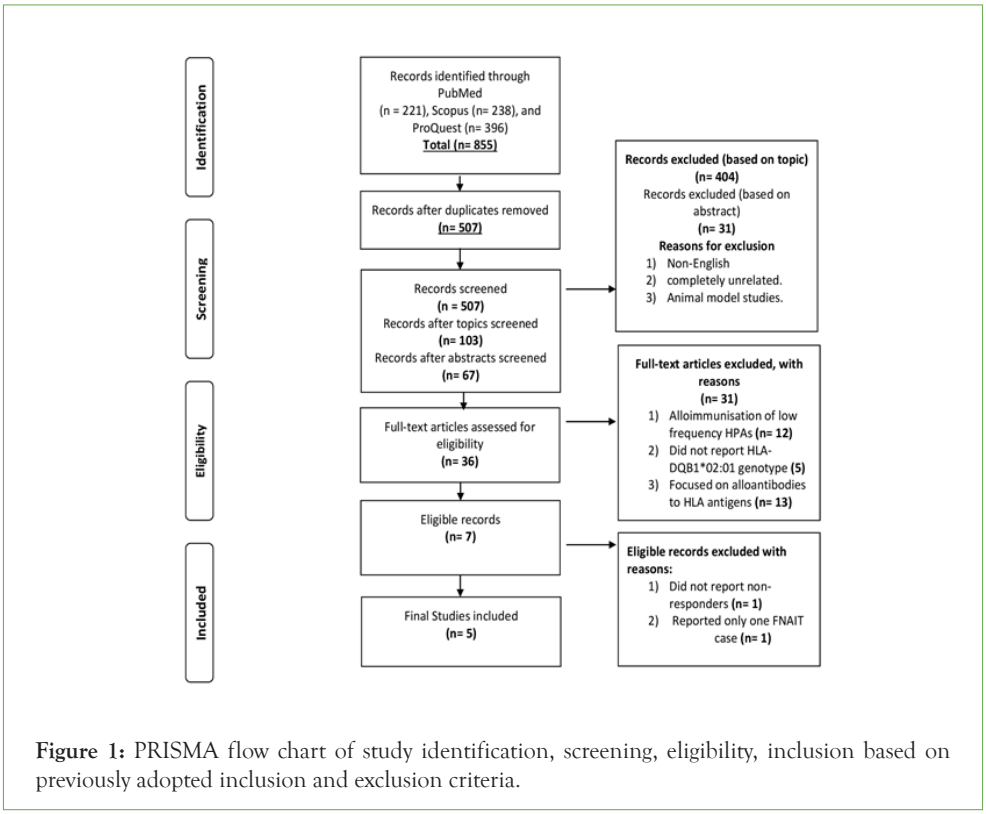

Figure 1: PRISMA flow chart of study identification, screening, eligibility, inclusion based on previously adopted inclusion and exclusion criteria.

Table 1: Summary of the eligible data from included studies [8, 11, 37-39].

			Responders			Non-responders (control)		
Study	Country	Study design	Total responders	possessing HLA-DQB1*02:01	%	Total non-responders	possessing HLA-DQB1*02:01	%
L'Abbe et al. 1992 [8]	Canada	Retrospective	36	34	94%	10	3	30%
Delbos et al. 2016 [11]	France	Retrospective	45	29	64%	19	6	32%
Loewenthal et al. 201 [37]	Israel	Retrospective	23	17	74%	24	9	37%
Sainio et al. 2017 [38]	Finland	Retrospective	71	51	78%	25	10	40%
Sukati et al. 2005 [39]	Scotland	Retrospective	14	12	85%	7	1	14%

Table 2: Assessment of the quality of included studies according to STROBE checklist.

Study	Sample size: was it predetermined?	Sample size; acceptable level of significance	Patient eligibility and selection	The genotype of all patients for HLA-DRB3*01:01 was reported	The genotype of all patients for HLA-DQB1*02:01 was reported	Clear reporting of alleles genotyping in the result section	Tests performed before FNAIT diagnosis	Adequate description of study populations	Clear explanation of the association of HLA-DRB3*01:01 with FNAIT	Clear explanation of the association of HLA-DQB1*02:01 with FNAIT
L'Abbe et al. 1992 [8]	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Delbos et al. 2016 [11]	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No
Loewenthal et al. 2013 [37]	No	Yes	Yes	Yes	Yes	Yes	No	No	Yes	No
Sainio et al. 2017 [38]	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No
Sukati et al. 2005 [39]	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No

Note: Yes=criteria fulfilled; No=criteria not fulfilled.

Table 3: Inheritance of DQB1*02:01 in responders (FNAIT cases) and non-responders.

	HLA DQB1*02:01			HLA DRB3*01:01		
Study	In responders	In non-responders	Odds ratio	In responders	In non-responders	Odds ratio (95% CI)
L'Abbe et al. 1992 [8]	94% (34/36)	30% (3/10)	39.7	91% (32/35)	20% (2/10)	42.7
Delbos et al. 2016 [11]	64% (29/45)	32% (6/19)	3.9	84% (38/45)	21% (4/19)	20.4
Loewenthanl et al. 2013 [37]	74% (17/23)	37% (9/24)	4.7	87% (20/23)	2% (2/24)	73.3
Sainio et al. 2017 [38]	78% (51/71)	40% (10/25)	3.8	100% (71/71)	52% (13/25)	132.4
Sukati et al. 2005 [39]	85% (12/14)	14% (1/7)	36.0	93% (13/14)	14% (1/7)	78.0
Mean	76% (143/189)	34% (29/85)	6.600	93% (174/188)	26% (22/85)	41.429

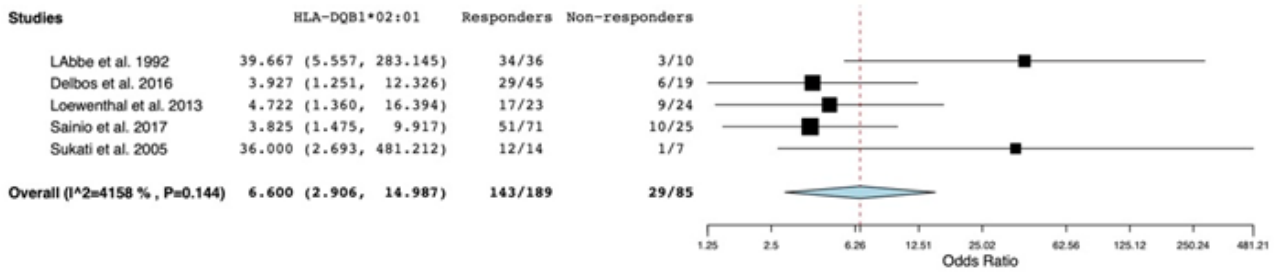

Figure 2: Forest plot of the inheritance of HLA-DQB1*02:01 in responders and non-responders and its association with FNAIT. Responders: HPA-1bb mothers who had babies with confirmed FNAIT due to anti-HPA-1a antibodies. Non-responders: HPA-1bb mothers who had been pregnant with HPA-1ab but did not undergo any complications to their newborns. C.I.: Confidence Interval. The size of the squares in the forest plots indicates the number of responders and non-responders, the bigger the box, the greater number of participants in the study. The location of each square indicates the odds ratio for the study that belongs to that square. The dashed line represents the odds ratio mean which was 6.60.

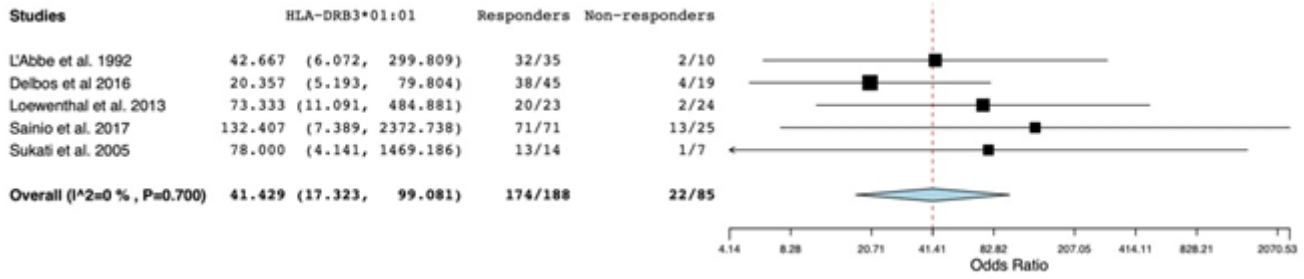

Figure 3: Forest plot of the inheritance of HLA-DRB3*01:01 in responders and non-responders and its association with FNAIT. Responders: HPA-1bb mothers who had babies with confirmed FNAIT due to anti-HPA-1a antibodies. Non-responders: HPA-1bb mothers who had been pregnant with HPA-1ab but did not undergo any complications to their newborns; C.I.: Confidence Interval. The size of the squares in the forest plots indicates the number of responders and non-responders, the bigger the box, the greater number of participants in the study. The location of each square indicates the odds ratio for the study that belongs to that square. The dashed line represents the odds ratio mean which was 41.42.

The pattern of the inheritance of HLA-DRB3*01:01 and HLA DQB1*02:01 alleles

The inheritance of HLA-DRB3*01:01 in responders and non-responders who lack HLA-DRB3*01:01. HLA-DRB3*01:01 was not possessed by three responders in to L'Abbe et al. study [8]. These three responders who lacked HLA-DRB3*01:01 were found to have HLA-DQB1*02:01. Similarly, one responder in Sukati et al. study lacked HLA-DRB3*01:01 and possessed HLA-DQB1*02:01. In contrast to Loewenthal et al. study, the three responders who lacked HLA-DRB3*01:01 were found to also lack HLA-DQB1*02:01. In L'Abbe et al., eight out of ten non-responders lacked HLA-DRB3*01:01 and also lacked HLA-DQB1*02:01 while two non-responders lacked both alleles. Nine of 22 negative-HLA-DRB3*01:01 non-responders in Loewenthal et al. were found to possess HLA-DQB1*02:01. In the same study, Loewenthal et al., out of the total number of non-responders, 12 women were found to lack both alleles. In Sukati et al., only one of the six non-responders who lacked HLA-DRB3*01:01 was found to possess HLA-DQB1*02:01. These results suggest that the inheritance of both alleles is most likely to be independent (Table 4) [8,37,39] .

Table 4: The inheritance of HLA-DQB1*02:01 in HLA-DRB3*01:01 negative responders and non-responders.

	Responders					Non-responders				
study	Total responders tested for HLA-DRB3*01:01	No. of responders possessing HLA-DRB3*01:01	No. of responders lacking HLA-DRB3*01:01	No. of HLA-DQB1*02:01 in responders who lack HLA-DRB3*01:01	Lack both alleles	Total non-responders tested for HLA-DRB3*01:01	No. of non-responders possessing HLA-DRB3*01:01	No. of non-responders lacking HLA-DRB3*01:01	No. of HLA-DQB1*02:01 in non-responders who lack HLA-DRB3*01:01	Lack both alleles
L'Abbe et al. 1991 [8]	35	32 (91%)	3	3	0	10	2 (20%)	8	0	2
Delbos et al. 2016 [11]	45	38 (84%)	7	NR	NR	19	4 (21%)	15	NR	NR
Loewenthanl et al. 2013 [37]	23	20 (87%)	3	0	3	24	2 (8%)	22	9	12
Sainio et al. 2017 [38]	71	71 (100)	0	NA	0	25	13 (52%)	12	NR	NR
Sukati et al. 2005 [39]	14	13 (93%)	1	1	0	7	1 (14%)	6	1	4

Note: No.: Number. NR: Not Reported. NA: Not Applicable.

DISCUSSION

For many years, numerous studies have investigated the allelic variants of HLAs to determine their predictive value for FNAIT detection. There is a long-known correlation between HLA-DRB3*01:01 and foetal HPA-1a alloimmunisation in FNAIT. However, the aim of this systematic review and meta-analysis was to investigate the association of HLA-DQB1*02:01 with confirmed FNAIT cases (responders). A total of five retrospective studies were considered eligible as they fit the adopted inclusion and exclusion criteria. As it is mentioned earlier, responders are HPA-1bb mother who had babies with confirmed FNAIT due to anti-HPA-1a antibodies, and non-responders were HPA-1bb mothers who had been pregnant with HPA-1ab but did not undergo any complications to their newborns [8,11,37-39]. Thus, we extracted two study groups that aligned with the aim of the study, responders and non-responders, to statistically investigate the implication of HLA-DQB1*02:01 in FNAIT. The percentages of HLA-DQB*02:01 were high in responders of all included studies compared to non-responders. Overall, this allele was associated with 76% of all included responders compared to 34% of non-responders. These results suggest an evident correlation between HLA-DQB1*02:01 and FNAIT condition. Our study also confirms the long-known concept that there is a strong association of HLA-DRB3*01:01 with FNAIT cases. This strong association was expected. It is well known that HLA-DRB3*01:01 plays a significant role in mounting the immune response especially if the peptide was from HPA-1a [37,42]. The high avidity of the hydrophobic cavity of HLA-DRB3*01:01 molecule to HPA-1a peptides is believed to be the main reason of the high frequency of this allele in FNAIT mothers.

Overall, the average percentage was 93% of all responders reported in the included five studies (OR=41.429, P-value ≤ 0.001). Despite this high outcome, HLA-DRB3*01:01 was not found in all positive FNAIT cases which suggests that this allele does miss some confirmed FNAIT cases. It is true that the percentages of HLA-DQB1*02:01 in responders were not as high as those of HLA-DRB3*01:01, but HLA-DQB1*02:01 was found in some responders who lack HLA-DRB3*01:01. This might further promote the predictive value for testing both HLA-DRB3*01:01 and HLA-DQB1*02:01 alleles.

Interpretation of the overrepresentation of HLA-DQB1*02:01 in FNAIT

The association of HLA-DQB1*02:01 with FNAIT is shown by the high frequency of this allele in responders. Two studies hypothesised that this association might be due to linkage disequilibrium with HLA-DRB3*01:01 [11,43]. We noticed that the inheritance of either alleles or one of them did not show any consistent linkage based on the provided data of the five included studies. For example, the three responders in L'Abbe et al., who lack HLA-DRB3*01:01 were found to possess HLA-DQB1*02:01; however, in Leowenthanl et al., the responders who lack HLA-DRB3*01:01 were found to also lack HLA-DQB1*02:01; additionally, some responders lack both alleles. Therefore, the hypothesis that states that the overrepresentation of HLA-DQB1*02:01 maybe due to linkage disequilibrium is highly questionable [8,37]. These observations strongly indicate that there is independent involvement of both alleles, HLA DRB3*01:01 and HLA DQB1*02:01, with FNAIT.

The importance of HLA-DQB1*02:01 and the possibility of using it as another risk factor

Table 4 shows the obvious independent inheritance of both alleles. Despite significantly lower percentages of FNAIT in HLA-DRB3*01:01 negative mothers, in the included five studies, a total of 13 HLA-DRB3*01:01 negative women developed anti-HPA-1a antibodies and then FNAIT to their newborns/fetuses [8,11,37-39]. Thus, not all positive FNAIT cases (responders) possessed HLA-DRB3*01:01. For example, in L'Abbe et al., HLA-DRB3*01:01 was found in 32 out of 35; this means that three responders did not possess HLA-DRB3*01:01 [8]. Interestingly, these three responders possess HLA-DQB1*02:01. This brings us to the idea that HLA-DQB1*02:01 allele could be used as a complementary risk factor for FNAIT disorder. This also applies to Sukati et al. study where the only responder who lacks HLA DRB3*01:01 allele was found to possess HLA DQB1*02:01 allele [39]. However, because of the relatively small number of non-responders, larger simple size studies are needed to further investigate the possibility of using HLA-DQB1*02:01 allele as a predictive factor in parallel with HLA DRB3*01:01 allele.

Strengths and limitations

Our study is the first systematic review and meta-analysis that demonstrate the importance of HLA-DQB1*02:01 in foetal and neonatal alloimmune thrombocytopenia. For years, it is well known that FNAIT cases that caused by anti-HPA-1a were mostly found to be HPA-1bb mothers possessing HLA-DRB3*01:01. Therefore, HLA-DRB3*01:01 allele was a very important tool used as a stratification risk factor for FNAIT conditions [8,37,39]. However, HLA-DQB1*02:01 was also associated but with lower percentages compared to HLA-DRB3*01:01 allele. Based on our observations in this systematic review, the inheritance of these two alleles did not show any consistency nor linkage and thus the possibility of using HLA-DQB1*02:01 as a complementary predictive factor for FNAIT is another strength in our study.

As we needed to compare HPA-1bb mother who developed FNAIT (responders) to non-responders, a lot of studies that investigated FNAIT were excluded as they compare their outcomes with general population. This was considered too broad as general population involves males, women with unknown history of FNAIT and women with normal history of pregnancies. In addition, we made sure that all responders must be all immunised against foetal HPA-1a and not other foetal platelet antigen. This is a crucial reason why we found only five studies that fit all inclusion and exclusion criteria. Furthermore, despite covering FNAIT that is caused by HPA-1a antibodies as the main cause of FNAIT, there is still 7-16% of FNAIT cases were found to be caused by HPA-5b inherited in foetus from father, and 2-4% of FNAIT cases were caused by maternal alloantibodies against foetal HPA-15b [4,15,44,45]. In these particular cases, it is believed that HPA-1bb 5aa mothers who lack HLA-DRB3*01:01 cannot make anti-HPA-1a antibodies even if they were pregnant with HPA-1ab foetus [14,23]. In addition, ethnicity is another aspect that should be considered as it might play a role in the occurrence of FNAIT. For example, the incidence of HPA-1a induced FNAIT is lower in African American mothers; this population, however, has high risk of developing alloantibodies against foetal HPA-2 and HPA-5 antigens [14,46]. Another example is the Japanese population; it is found that HPA-4 and HPA-5 were the most implicated foetal platelet antigens to cause FNAIT [14,38,47]. Thus, one of the limitations in this systematic review is that there are some other factors that play a crucial role in the incidence of FNAIT. The small number of non-responders in all studies compared to responders had made some limitations when extracting data and interpreting outcomes. In Table 4, we wanted to obtain the number of HLA-DQB1*02:01 in responders who lack HLA-DRB3*01:01, but in one study, all responders were found to possess HLA-DRB3*01:01 and this was not applicable [38]. The same study and other study did not report the number of non-responders who lack both alleles, HLA DRB3*01:01 and HLA-DQB*02:01 [11,38]. Moreover, Delbos et al. study, did not report the number of HLA-DQB1*02:01 in responders who lack HLA-DRB3*01:01 [11]. Although this was not a priority in our study nor it was in the adopted inclusion and exclusion criteria, it would have been useful to broadly demonstrate our second hypothesis of using HLA-DQB1*02:01 as a complementary risk factor [48].

For future studies, we suggest that HLA-DQB1*02:01 should be focused on as a very crucial allele. As it is confirmed in this review that HLA-DQB1*02:01 can be possessed by HPA-1bb responders who lack HLA-DRB3*01:01, we suggest that genotyping for HLA-DQB1*02:01 might assist to further predict the incidence of FNAIT. Although it is known that there is a high hydrophobic cavity of HLA-DRB3*01:01 to bind HPA-1a peptides, the hydrophobic status of HLA-DQB1*02:01 molecule is still unknown. Therefore, we suggest that addressing this gap in future studies would be beneficial.

CONCLUSION

This systematic review/meta-analysis includes five eligible studies which were reviewed carefully to extract related data. All five studies showed an acceptable fulfilment of STROBE checklist. The study design for all five studies was retrospective. Human leukocyte antigens (HLA class II) were found to be implicated in the presentation of HPA-1a peptides with different avidity. HLA-DRB3*01:01 is believed to have a high hydrophobic cavity to bind peptides of HPA-1a. HLA-DRB3*01:01 and HLA-DQB1*02:01 alleles were strongly associated with FNAIT. The overrepresentation of HLA-DQB1*02:01 may be independent. The only explanation for this allelic overrepresentation is that HLA-DQB1*02:01 seems to play an important role in the incidence of FNAIT due to HPA-1a antibody. The hydrophobic cavity of HLA-DQB1*02:01 should be investigated in future studies. HLA-DQB1*02:01 might be used as a complementary risk factor if confirmed to be independently inherited.

DECLARATION

Conflict of interest

Authors declare that there is no conflict of interest.

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