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ORIGINAL ARTICLE

Gene expression in obstetric antiphospholipid syndrome: a systematic review

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Abstract

Background: Antiphospholipid syndrome (APS) is a multisystem disease that may present as venous or arterial thrombosis and/or pregnancy complications with the presence of antiphospholipid antibodies. Until today, heterogeneity of pathogenic mechanism fits well with various clinical manifestations. Moreover, previous studies have indicated that genes are differentially expressed between normal and in the disease state. Hence, this study systematically searched the literature on human gene expression that was differentially expressed in Obstetric APS. *Methodology:* Electronic search was performed until 31st March 2015 through PubMed and Embase databases; where the following Medical Subject Heading (MeSH) terms were used and they had been specified as the primary focus of the articles; gene, antiphospholipid, obstetric, and pregnancy in the title or abstract. From 502 studies retrieved from the search, only original publications that had performed gene expression analyses of human placental tissue that reported on differentially expressed gene in pregnancies with Obstetric APS were included. Two reviewers independently scrutinized the titles and the abstracts before examining the eligibility of studies that met the inclusion criteria. For each study; diagnostic criteria for APS, method for analysis, and the gene signature were extracted independently by two reviewers. The genes listed were further analysed with the DAVID and the KEGG pathways. *Results:* Three eligible gene expression studies involving obstetric APS, comprising the datasets on gene expression, were identified. All three studies showed a reduction in transcript expression on *PRL*, *STAT5*, *TF*, *DAF*, *ABCA1*, and *HBEGF* in Obstetric APS. The high enrichment score for functionality in DAVID had been positive regulation of cell proliferation. Meanwhile, pertaining to the KEGG pathway, two pathways were associated with some of the listed genes, which were ErBb signalling pathway and JAK-STAT signalling pathway. *Conclusion:* Ultimately, studies on a genetic level have the potential to provide new insights into the regulation and to widen the basis for identification of changes in the mechanism of Obstetric APS.

Keywords: antiphospholipid, gene, obstetric, pregnancy

INTRODUCTION

Antiphospholipid syndrome (APS) is a multisystem autoimmune disorder that may present as venous or arterial thrombosis and/or pregnancy complications in association with the presence of circulating antiphospholipid antibodies. APS is generally divided into two different types, which are primary and secondary APS. Primary APS, which is also known as isolated APS, appears without the occurrence of other autoimmune disorders, as opposed to the secondary form, where it is related to other autoimmune disorders, such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). In 1998, the first preliminary criteria for classification of APS were developed in Sapporo, Japan.[1] This classification of APS was achieved when the subject fulfilled at least one of the clinical manifestations with persistent positive

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laboratory criteria. In 2006, these criteria were then reviewed and updated and are now known as the Sydney criteria.[2] In the Sydney criteria, two modifications were added to the laboratory criteria, which are the extension of time between two positive determinations from 6 weeks to 12 weeks and the test for anti-β2 Glycoprotein 1 (β2-GP1) IgG and IgM. This is to ensure the detection of persistent antibodies only.[2] Generally in APS, a pro-thrombotic mechanism plays a role in the formation of blood clot, involving either the arterial wall or the venous bed.[3]

Furthermore, the disruption of the normal function of phospholipid leads to an imbalance in haemostatic environment, which in turn, leads to acquired hypercoagulability. Hypercoagulability of the blood is one of the known main consequences of APS in the general population.[4] The reason why anti-β2GP1 measurements were added in Sydney criteria was because researchers found that the autoantibodies in APS do not actually bind directly to the phospholipid itself but, rather prefers to bind to β2-GP1.[5] β2-GP1 molecule acts as an anticoagulant protein and is in a circular form naturally. When the β2-GP1 is exposed to anionic phospholipid, it binds to the domain V of β2-GP1. The anti- β2-GP1 or the autoantibodies bind to the domain I of β2-GP1, which is exposed when it is not in a circular form. The disruption and the dysfunction of β2-GP1 molecule lead to thrombosis.[6]

Additionally, in pregnant women, the APS could result in placental insufficiency, manifesting as obstetric complications, as well as tragic pregnancy loss in the form of miscarriages or intrauterine death, and even premature birth. The prevalence of APS was estimated to be 0.5% from that of the general population.[7] In cases of recurrent miscarriages, about 10-15% of women represented APS.[8] In addition to this, the presence of antibodies increased to 15% in women who had experienced recurrent first trimester losses.[9] Thus, obstetric APS is the focus of interest among many researchers. However, to date, researchers are still trying to identify the mechanism that contributes to the pathogenesis of this disorder. The suggested mechanisms are an intra-placental thrombotic phenomenon, an inflammatory process, and a direct antiphospholipid mediated damage, all of which cause disruptions in implantation and placentation of the embryo.[10] These mechanisms suggested are not mutually exclusive and may occur either in isolation or in combination at different gestational stages of pregnancy.

On top of that, many other clinical disorders need to be ruled out when managing cases of Obstetric APS. Different clinical outcomes suggest that more than one mechanism is involved in Obstetric APS. This ranges from being totally asymptomatic to patients experiencing early to late miscarriages and pre-eclampsia.[8] Heterogeneity in the outcome of this disorder would usually result in delayed identification and diagnosis. To date, the main mechanisms that have been outlined and studied by researchers include thrombosis, inflammation, impairment of implantation and complement activation.[11] However, the exact mechanism that contributes to Obstetric APS still remains unclear.

Measurement in gene expressions has been used to develop new biological concepts, refine disease classification, improve diagnostics and prognostic accuracy, as well as identify new molecular targets for drugs.[12] The potential results and findings from these measurements are commonly reported in the form of a list of genes that are differentially expressed between normal and diseased patients or that correlate with different prognosis or phenotypes. In this study, the literature on gene expression studies in obstetric APS was systematically reviewed in order to determine a common gene expression signature. By studying the gene expression, we could then potentially provide new insights into the regulation of genes in patient with Obstetric APS. This study offers new knowledge in explaining the basis of identification of the changes towards the mechanism attributed by antiphospholipid antibodies in Obstetric APS.

METHODS

Data sources and search strategy

This systematic review was performed based on the Preferred Reporting Items for Systematic Reviews (PRISMA). An electronic search, by using PubMed and Embase, was employed to identify relevant research publications with an unlimited starting publication date until 31st March 2015. The search strategy further applied a combination or broad indexing terms of each database. The following Medical Subject Heading (MeSH) terms were used and these keywords were specified to become the primary focus of the articles; antiphospholipid, gene, obstetric, and pregnancy in the title or abstract. Additional MeSH and text terms were identified by reviewing available review articles. These

included antibodies, Hughes Syndrome, placenta, and pregnant. Other than that, additional references were also identified after reviewing the bibliographies of the retrieved studies.

Inclusion and exclusion criteria

Only English-language publications were included for screening, while publication types without primary data, such as letter to editors, editorials, case reports, conference proceedings, and narrative reviews, were excluded. Studies that involved non-obstetric APS and in-vitro studies or also known as induced APS were also excluded. To be eligible for the review, the research publications must be experimental studies on samples from human tissue or blood of obstetric APS cases that had performed gene expression analysis. Information was extracted from each study for; (1) characteristics of study design, study objective, population, and method of gene expression analysis; (2) there must be no interventions and limited to observational study; (3) comparison between patients of obstetric APS and normal patients; (4) outcome measurements on the gene expression being upregulated and downregulated in Obstetric APS; as well as (5) criteria defining APS.

Identification of Relevant Studies and Data Extraction

All manuscripts from searchers were downloaded into an EndNote library (X5.0.1). Potentially relevant papers were selected by screening the titles, the abstracts, and the entire articles retrieved from the database searchers. First, two reviewers independently scrutinized the titles and the abstracts of all retrieved references to select potentially eligible articles. The duplication of papers was discarded by EndNote Software. Full text papers references selected by at least one reviewer were obtained. Second, the two reviewers examined these full text papers to determine if they met the predetermined inclusion criteria. Disagreements on inclusion were resolved by consensus through discussion. Records on reasons for rejection were kept. Data were extracted from the included studies into a table. The details of each of the studies, including study design, sample size, sampling type, method for gene analysis, and genes identified, were extracted and listed in the table. Data entry was also checked for each study after completing data extraction.

Study quality and analysis

The research quality was assessed by discussing reported details of analysis amongst the authors. The authors focused on the results and the reported list of gene expressions. In order to ascertain the validity of eligible studies, pairs of reviewers worked independently and with adequate reliability. For each study, bias was excluded by adhering to the inclusion criteria. The genes listed were further analysed with the Database for Annotation, Visualization, and Integrated Discovery (DAVID).[13] Further analysis was performed to determine the cluster of genes that displayed significant functional annotation enrichment and those enriched annotations could be related to the Obstetric APS, while the contribution of genes in the pathway was based on the Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway.[14]

RESULTS

The database search identified 502 titles that were potentially relevant. The EndNote software (X5.0.1) was used to identify and remove duplicates. A total of 431 articles had been retrieved for abstract review (Fig. 1). Screening the titles and the abstracts resulted in the selection of nine potentially relevant citations for full text review. However, six of these articles did not fulfil the inclusion criteria, hence, leaving only three articles for analysis.

Only these three publications provided univariate data on the gene expression in cases of obstetric APS. Table 1 summarizes the characteristics of these studies. Homogeneity of studies based on study design was needed to prevent bias as in the intervention study that could interfere with the selection of cases of Obstetric APS. This was achieved through the use of a stringent inclusion criterion that had to be fulfilled. Thus, all studies that were retrieved had the same study design, which was observational study. All studies were heterogeneous with respect to the study objectives. A study by Francis looked into whether impaired endometrial differentiation before conception contributed to the pregnancy complications in APS.[15] In another study by Albrecht, the main objective was to investigate if ATP-binding cassette transporter (*ABCA1*) mRNA was altered in placentas from pregnancies complicated by APS.[16] Meanwhile, a study carried out by Di Simone determined if abnormal Heparin Binding-Epidermal Growth Factor (*HB-EGF*) expression played a role in

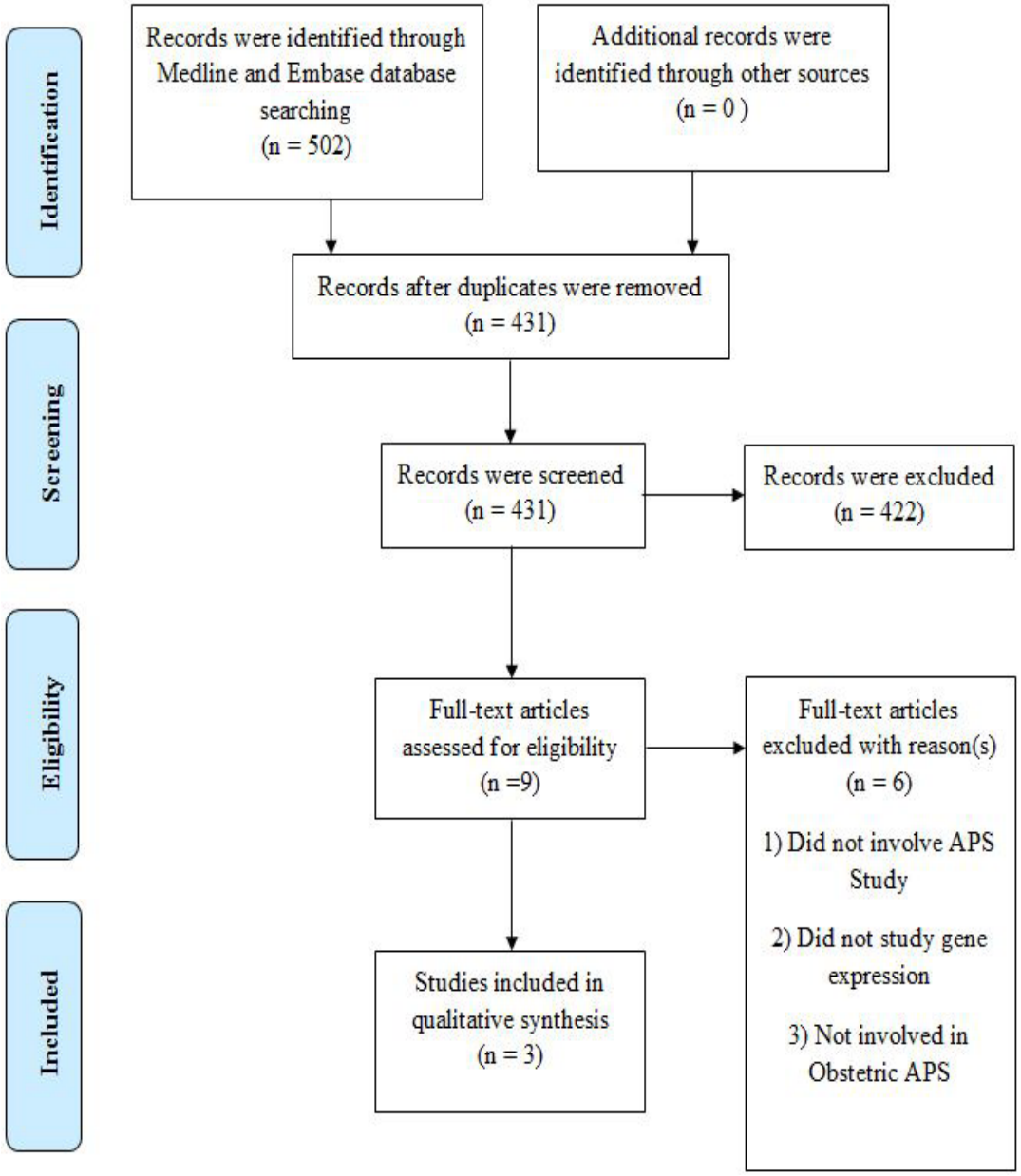

FIG. 1: Workflow of the selection

antiphospholipid antibody mediated by defective placentation.[17] In fact, two studies used the placenta from patients of Obstetric APS,[16,17] while another study used the endometrial sample obtained from a cohort of women with recurrent pregnancy losses.[15] All these studies utilized both the clinical and the laboratory criteria for APS based on the Sapporo criteria.

Gene expression analysis

Gene expressions can be analysed through many platforms, such as Polymerase Chain Reaction, Microarray, and Gene Sequencing. Quantitative Real-Time PCR (qRT-PCR) was used in all studies as a platform for analysis.

Gene expression from all studies revealed different genes with different functions. A study conducted by Francis involved the expression of certain endometrial differentiation markers and complement regulatory proteins. Endometrial differentiation markers, such as Prolactin (*PRL*), Tissue Factor (*TF*), and Signal Transducer and Activator of Transcription 5 (*STAT5*), showed significant reductions. The

TABLE 1: Data extracted from the various retrieved studies*

First Author & Year	Sample	Method	Results
Francis *et al,* 2006	Endometrium Sample	• Quantitative RT-PCR	• The expressions of certain endometrial differentiation markers, such as **Prolactin (*PRL*), Tissue Factor (*TF*), Signal transducer, and activator of transcription 5 (*STAT5*)** decreased significantly. • Gene expression of **Decay Accelerating Factor (*DAF/CD55*)**, which is a marker of Complement Regulatory Proteins, also decreased significantly.
Albrecht *et al*, 2007	Placenta	• Quantitative RT-PCR	• **ATP-binding cassette transporter A1 (*ABCA1*)** mRNA expression was significantly reduced in the placentas from women with APS.
Di Simone *et al,* 2010	Placenta	• Quantitative RT-PCR	• Reduction in **Heparin-binding epidermal growth factor (*HBEGF*)** expression.

*The extraction had been based on Author, Year, Method, Type of Sample, and Results that portrayed the signature of gene expression

complement regulatory proteins showed that Decay Accelerating Factor (*DAF*) expression also decreased significantly. The other two studies by Albrecht and Di Simone revealed a single gene each, namely *ABCA1* and *HB-EGF*, respectively. Both genes showed reductions in gene expression in Obstetric APS.

The overall enrichment score of 4.08 was based on the Expression Analysis Systematic Explorer (EASE) score of each term member in annotation cluster 1 (Table 2). The positive regulation of cell proliferation exhibited the highest enrich term due to the lowest *p*-value compared to the other two terms, which are regulation of cell proliferation and regulation of cell growth. Positive regulation of cell proliferation involved *TF*, *HBEGF*, *PRL*, *STAT5A*, and *STAT5B* genes. Based on the KEGG pathway, two pathways were involved in at least two of the listed genes, which were Avian Erythroblastosis Oncogene B (ErBb) signalling pathway and Janus Kinase-Signal Transducer and Activator of Transcription (JAK-STAT) signalling pathway (Fig. 2). *PRL* and *STAT5* were both involved in JAK-STAT signalling pathway, while another pathway showed the involvement of *HBEGF* and *STAT5* in ErBb signalling pathway (Fig. 3).

DISCUSSION

At present, the mechanism of APS still remains unclear. Gene expression studies which were identified in this review support the involvement of genes that plays a role in implantation, coagulation, complement regulation and steroidogenesis in the placenta. Despite of

TABLE 2: Gene Ontology Term based on EASE score*

Gene Ontology Term	p-value	Benjamini value
Positive regulation of cell proliferation	1.2×10^{-5}	5.3×10^{-3}
Regulation of cell proliferation	1.6×10^{-4}	1.3×10^{-2}
Regulation of growth	3.0×10^{-4}	1.8×10^{-2}

*Overall enrichment score based on EASE score in terms of annotation cluster is 4.08. Based on the Gene Ontology (GO) term, the highest enrich term was discovered in positive regulation of cell proliferation. The significant value was $p < 0.05$[13]

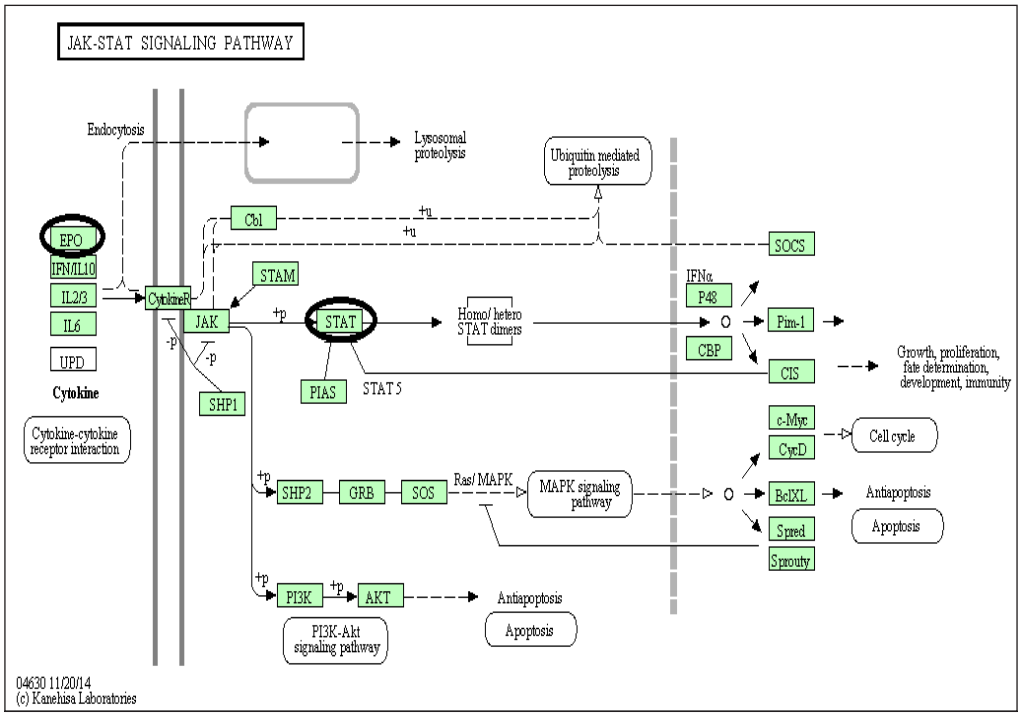

FIG. 2: JAK-STAT signalling pathway generated by the KEGG pathway. STAT5 is a gene involved in JAK-STAT signalling pathway. This pathway may end up with various processes, such as cell cycle and apoptosis.[14] Reproduced with permission from KEGG.

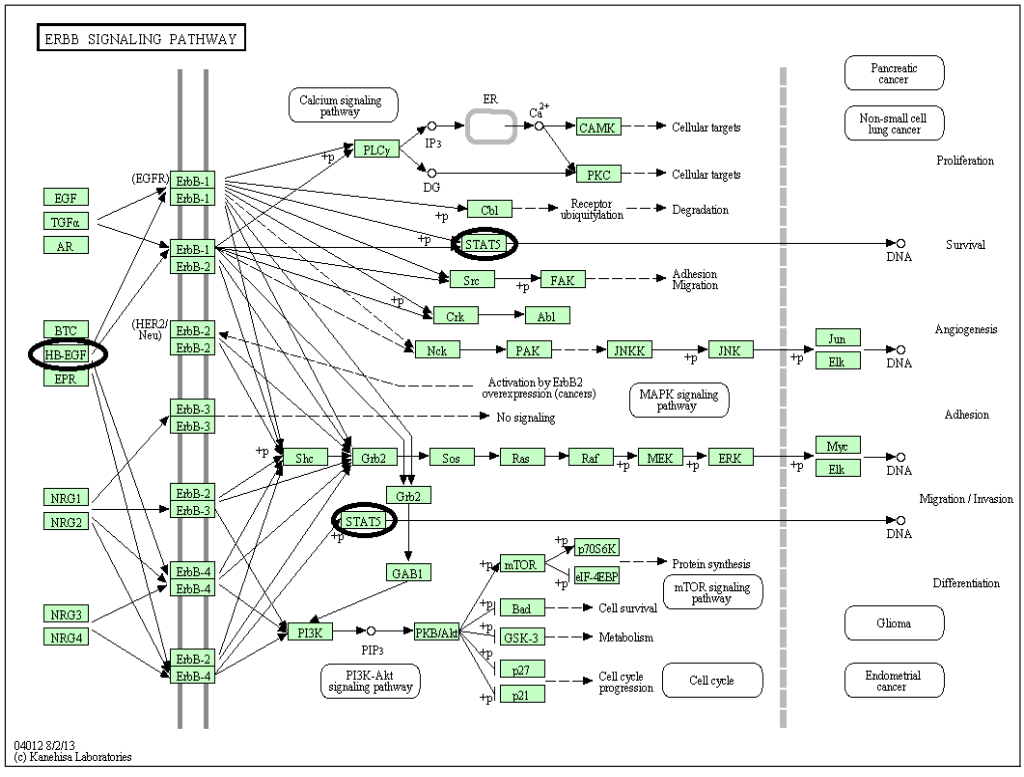

FIG. 3: ErBb signalling pathway generated by KEGG pathway. HBEGF and STAT5 play a role in ErBb signalling pathway. HBEGF is a ligand that is bound to various ErBb protein receptors.[14] Reproduced with permission from KEGG.

the limitations in the number of the available studies, six genes; *PRL*, *TF*, *STAT5*, *ABCA1*, *DAF*, and *HBEGF*, were found to be reduced in their expressions and were downregulated in the mechanisms that involved Obstetric APS. These genes were also identified to play a role in the outcome of pregnancies with APS.

Nonetheless, the study carried out by Francis was the first to show that the gene expression of endometrial markers; *PRL* and *TF*, decreased in Obstetric APS.[15] *PRL* is a protein that was first to be synthesized from human endometrium and decidua in 1977.[18] *PRL* is one of the ligands that bind to cytokine receptor in the JAK-STAT signalling pathway.[19] The final stage of the cascade activation results in the growth, proliferation, fate determination, development, and immune state of the foetus.[20] The function of *PRL* in pregnancy is crucial for implantation and early pregnancy. This statement is supported by in-vitro observation that have shown *PRL* can stimulate cell migration and invasion of human trophoblast.[21] Moreover, case control studies have also reported that the expression of *PRL* was impaired or absent in the villi of women with spontaneous first trimester miscarriage.[22] However, a study reported that hyperprolactinemia (*HPRL*) was associated with pregnancy complications, such as early and late pregnancy losses, as well as intrauterine growth retardation.[23] Between these two studies, differences were found in *PRL* expression and this is most likely due to the variability in study design. The low levels of PRL expression in early pregnancy may well be a contributing factor that causes the unsuccessful implantation in patients with Obstetric APS.

TF expression was also shown to decrease in Obstetric APS.[15] Generally, *TF*, which is known as thromboplastin, is the key initiator of the coagulation cascade. It binds factor VIIa, resulting in activation of factor IX and factor X, ultimately leading to a burst in thrombin that initiates and sustains the formation of fibrin.[24] The data also suggested that increased *TF* activity is an important cause of hypercoagulability in APS.[25] Antiphospholipid antibodies in APS activate the monocytes, the platelets, and the endothelial cells, which cause the overexpression of *TF*. This overexpression causes the hypercoagulability state and may provoke the termination of pregnancy.[26] Studies that involved autoantibodies, specifically anti-β2-GP1, triggered an increase in expression of *TF* on monocytes and vascular endothelial cells by increasing their expression.[27] Function of *TF* as the main player initiating coagulation is crucial in pregnancy, especially in the first trimester, which aims to prevent haemorrhage during implantation.[28] Hence, the unregulated expression may cause thrombosis and inflammation process to occur in patients suffering from Obstetric APS; resulting in unsuccessful implantation, and therefore, miscarriage. Furthermore, many previous studies have stated that *HPRL* and *TF* are mostly found in pregnancy complications and these are different from that obtained in the study done by Francis. This may be due to the fact that the samples from recurrent pregnancy loss among women with APS were obtained before conception. The most likely scenario is that the expression of *TF* and *PRL* varied throughout the different gestational stages of pregnancy.

In this review, one member of *STAT* was reported, which was *STAT5*. *STAT5* plays a pivotal role in cellular processes, such as proliferation, differentiation, and apoptosis.[29] *STAT5* consists of *STAT5A* and *STAT5B*. *STAT5* is involved in both pathways listed in the KEGG pathway, which are ErBb and JAK-STAT signalling pathways. Hence, it is essential for the expression of decidual marker genes in human endometrial stromal cells. An in vitro study using the monoclonal antibody, ID2 which exhibits a similar reactivity as human antiphospholipid, showed that the significantly reduced abundance of nuclear *STAT5* was associated with a reduction of *PRL* secretion and *PRL* promoter activation.[30] On the other hand, another study showed that the activation of *STAT5* by epidermal growth factor was responsible in regulation of cell proliferation and invasion of trophoblast cells for implantation of the embryo.[31] These various studies suggested that *STAT5* is indeed crucial for implantation and the dysregulation of *STAT5* expression could result in early pregnancy loss among women with Obstetric APS.

The expression of *DAF* was also reduced in Obstetric APS patients.[15] *DAF* or *CD55* is a complement regulatory protein. It prevents cell damage by interfering with the cascade through its binding with the complement proteins.[32] *DAF* is also one of the inhibitors of Complement 3 (C3) convertase other than the Complement Receptor-Related Gene Y (CRRY), Monocyte Chemoattractant Protein (MCP), and Inositol Phosphate (IP). Inhibition of C3 convertase decreases the formation of C5 in the making of the membrane attack complex prior to cell lysis. In addition, Iborra *et al*, revealed that under stress

conditions, high expression of *DAF* could protect the endometrium from complement-mediated lysis.[33] A study conducted by Banadakoppa showed that the reduction of *DAF* mRNA expression occurred in decidua obtained from patient who experienced spontaneous abortion.[34] Another study also suggested that decreased mRNA levels of *DAF/CD55* increase the vulnerability to complement-mediated cell damage during trophoblast invasion and placenta formation.[15] Therefore, it would seem that the reduction of *DAF/CD55* could also contribute to the unsuccessful implantation during the first trimester of pregnancy among patients suffering from Obstetric APS.

The *ABCA1* gene expression was also shown to be decreased in obstetric APS.[16] *ABCA1* gene plays a role in mediating cellular cholesterol and phospholipid efflux and it is involved in phosphatidyl-serine (PS) translocation and apoptosis. The metabolisms of placental cholesterol and lipid transport play fundamental roles in the survival and the development of the foetus.[35] PS translocation and apoptosis have a pivotal role in placental development and its regulation is crucial for a successful pregnancy.[36] A reduction of *ABCA1* proteins may influence the process of syncytialisation in human placental, which could lead to abnormal placentation.[36] Moreover, in murine study, it was shown that the loss of functional *ABCA1* caused severe placental malformation with structural abnormalities in the trophoblast part.[37] Therefore, abnormal placentation due to disturbance in the placental steroidogenesis could also contribute to early pregnancy loss or intrauterine growth restriction seen in women with Obstetric APS.

Finally, *HBEGF* is another gene, found to be reduced in Obstetric APS.[17] *HBEGF* is one of the activators for ErBb-1, ErBb-2, and ErBb-4 receptor proteins. The activation of these ErBb proteins, along with the activated *STAT5*, is pivotal for survival, migration, and invasion of cells. It is also involved in the action of the cascade in the calcium signalling pathway, Mitogen-activated protein kinase (MAPK) signalling pathway, Phosphoinositide 3-kinase-Protein kinase B (PI3K-Akt) signalling pathway, Myotonin protein kinase (MTPK) signalling pathway, and cell cycle.[38] *HBEGF* also binds to EGF Receptor, which is involved in the cascade pathway in the gene expression of the gonadotrophin secretion and plays a role in blastocyst implantation during early pregnancy. A decrease in *HBEGF* expression may lead to pregnancy complications, such as preeclampsia, due to unsuccessful implantation,[39] as well as intrauterine growth restriction due to elevated apoptosis.[40]

On top of that, the study generated by Albrecht demonstrated that mRNA and protein expression of ABCA1 were reduced in the placentas of women with APS, but not in isolated pre-eclampsia (PE).[16] These functional abnormalities may help explain the different pathologies between APS and PE. Moreover, the preliminary findings obtained by Di Simone suggest that the reduction of antiphospholipid-mediated HBEGF may be responsible for the defective placentation associated with APS.[17] The study carried out by Francis also showed that patient with recurrent pregnancy loss had well-defined endometrial gene expression, depending on the absence or the presence of circulating antiphospholipid antibodies. Hence, they suggested that impaired endometrial gene expression and lower DAF/CD55 before conception may compromise implantation and make vulnerable to complement-mediated pregnancy failure.[15] Based on the type of sample, only the study by Francis used endometrium as sample. The structural work was also very different from the other two studies as the study took into account the condition of patient before conception. Despite of the differences between these studies in terms of study design and study objective, studies on gene expression analysis had been important in explaining how the heterogeneity of genes contributed to various clinical manifestations found in Obstetric APS.

Limitations in data retrieved from this review had been largely contributed by the stringent criteria that had to be fulfilled, which was Observational study without treatment or cell or tissue induction. This ensured that the phenomenon of Obstetric APS occurred without being triggered by other factors. Many previous studies involved the induction APS either in vitro or in vivo. Thus, the interpretation of induced APS needs to be re-evaluated in observational studies in Obstetrics. Another limitation, which was related to the sample size amongst the three studies as sampling, was indeed challenging for all studies. Collection of genes that had been listed also showed some limitations. Not many researchers attempted to investigate gene expression in Obstetric APS. This may be attributed to the fact that obstetric APS is underdiagnosed, underreported, and defaulted follow-up. The addition of more clinical data

into the observation may also prove useful in determining the heterogeneity of the presentation and genes being expressed. Therefore, further studies should incorporate a system biology approach with functional genomics to further clarify when, how, and where these potential genes are activated and downregulated.

Conclusion

In conclusion, through this systematic review study, *PRL*, *TF*, *STAT5*, *ABCA1*, *DAF*, and *HBEGF* had been identified as candidate genes involved in the development of different mechanisms in the pathogenesis of Obstetric APS. Therefore, further experiments must be conducted on these candidate genes to study their expressions more extensively. The extension of gene expression analysis to protein expression analysis must be part of planning research that will offer explanation as to why and how the alterations of the genes have occurred. Ultimately, studies should have the potential to provide new insights into gene regulation and widen the basis for identification of changes in the mechanism that leads to different clinical manifestations in Obstetric APS.

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