

REVIEW

MECHANISMS IN ENDOCRINOLOGY

Proteomic biomarkers of type 2 diabetes mellitus risk in women with polycystic ovary syndrome

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Abstract

Women with polycystic ovary syndrome (PCOS) are at increased risk of developing insulin resistance and type 2 diabetes mellitus (T2DM). In this study, we attempted to list the proteomic biomarkers of PCOS and T2DM that have been published in the literature so far. We identified eight common biomarkers that were differentially expressed in both women with PCOS and T2DM when compared with healthy controls. These include pyruvate kinase M1/M2, apolipoprotein A-I, albumin, peroxiredoxin 2, annexin A2, α -1-B-glycoprotein, flotillin-1 and haptoglobin. These biomarkers could help improve our understanding of the links between PCOS and T2DM and could be potentially used to identify subgroups of women with PCOS at increased risk of T2DM. More studies are required to further evaluate the role these biomarkers play in women with PCOS and T2DM.

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Introduction

Polycystic ovary syndrome (PCOS) is a common endocrinopathy that affects 6–7% of women in reproductive age, according to three major epidemiological studies where classic diagnostic criteria were applied (1, 2, 3, 4). The modified diagnostic criteria (Rotterdam 2003) require two or more of the following: chronic ovulatory disorder (oligo-ovulation to anovulation and amenorrhoea), presence of hyperandrogenism manifested either in laboratory tests or in clinical symptoms and ultrasound evidence of polycystic ovaries where other causes of anovulation and hyperandrogenism have been ruled out (2, 5, 6). The pathogenesis of PCOS has been linked to the development of insulin resistance and hyperinsulinaemia, which often progress to long-term risks for type 2 diabetes mellitus (T2DM) with its associated micro- and macro-vascular complications (7, 8, 9).

A recent systematic review and meta-analysis showed that women with PCOS were four times more likely to develop T2DM compared with BMI-matched controls (10). Insulin resistance is proposed as a key pathophysiological feature of PCOS contributing to reproductive, cardiovascular and metabolic disturbances. It is suggested that women with PCOS often have more severe pathologically distinct insulin resistance than weight-matched non-PCOS populations (11, 12).

PCOS is also identified as a significant non-modifiable risk factor associated with T2DM by the International Diabetes Federation (13). There is, however, currently no way of determining which young women with PCOS will go on to develop T2DM in order to take appropriate preventative measures. An economic evaluation estimated that 40% of the economic costs of PCOS in the USA can be attributed to T2DM. This highlights the need for prevention of long-term complications through appropriate screening, diagnosis and intervention (14).

Proteomics is an emerging discipline that involves a large-scale study of the structure and function of proteins allowing the researcher to define protein expression changes in a single experiment (15). In a recent literature review of MEDLINE (1966–May 2012), EMBASE (1980–May 2012) and Cochrane (1993–May 2012) databases using the terms ‘proteomics’, ‘proteomic’, ‘type 2 diabetes’ and ‘PCOS’ or ‘PCOS’, no studies were identified in which proteomic techniques were used in PCOS women who also had T2DM. There were, however, studies in which proteomic techniques had been used in the study of T2DM and studies in which proteomic approaches had been applied to women with PCOS. The aim of this study was, therefore, to systematically review the research undertaken using proteomic methodologies for the identification of biomarkers in T2DM and identify whether there were

any potentially promising biomarkers for the detection of T2DM risk in women with PCOS by carrying out a search of these biomarkers against an updated previously published database of all proteomic biomarkers identified so far in women with PCOS (16). Any biomarker common to both conditions could, therefore, be investigated in further studies to understand the mechanisms linking T2DM with PCOS with a potential to facilitate screening by identifying women with PCOS who have high risk of developing T2DM.

Methodology

Institutional Review Board (IRB) approval was not obtained for this study as it was not considered necessary because the study did not involve patient contact. As a meta-analysis (quantitative analysis of the results) was not possible in this study, a heterogeneity evaluation was not warranted.

Studies eligible for review

MEDLINE (1966–May 2012), EMBASE (1980–May 2012) and Cochrane (1993–May 2012) databases were searched using the terms ‘proteomics’, ‘proteomic’ and ‘type 2 diabetes’. The inclusion criteria were studies that used proteomic technology in humans with T2DM. Animal studies or studies in which cell lines of patients with PCOS were investigated were excluded. To minimize bias and improve accuracy, this search was carried out independently by three of the authors (N G, T A and M X).

The original PDFs of studies obtained from the search were located through direct online links to the files from the search results. A manual search of references from all the studies was also conducted to identify any other potentially relevant studies. The search criterion ended by May 2012.

The main characteristics of the studies

The selected studies were screened and specific study characteristics were recorded. These included type of study design, number of participants (*n*) and population characteristics, type of proteomic technique used, focus of sample collected in each study (whether serum or saliva sample) and a list of proteins identified to have been expressed differently in patients with T2DM vs healthy controls was created. These parameters are presented in Table 1. To improve accuracy, screening of the studies was independently performed by three of the authors (N G, T A and M X).

Methodological quality assessment

The methodological quality of primary studies applying proteomics in T2DM was determined using the

QUADOMICS Tool, an adaptation of a quality assessment tool for use in systematic reviews of the diagnostic accuracy studies (QUADAS), which takes into account the particular challenges encountered in ‘-omics’-based techniques (17). The criteria that comprise the QUADOMICS Tool are shown in Fig. 1. Studies that achieved 10/16 or more on the QUADOMICS Tool were classified as high quality (HQ) whereas those that scored 9/16 or less were classified as low quality (LQ). The methodological quality assessment was also performed independently by three authors (N G, T A and M X).

Updating the PCOS proteomics database

The methods used to search for and abstract the data on the PCOS proteomic database have been previously published (16). An updated literature search was performed on MEDLINE (1966–May 2012), EMBASE (1980–May 2012) and the ISI web of knowledge (v4.2) databases using the following search terms ‘PCOS’ and ‘proteomic’, ‘proteomics’, ‘proteomic biomarker’ or ‘proteomics biomarker’ without any limits/restrictions. All relevant studies published since the previous database was published were reviewed. Thirteen papers (16, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29) were identified including four review articles and one study on mice. The review articles and the study on mice (21, 26, 27, 29) were excluded apart from a previous review article from our group in which original data on new biomarkers for PCOS had been published (16). A further three studies were abstracts from conference proceedings with no primary proteomic data on PCOS and so they were also excluded (22, 23, 24). Data from three of the remaining studies could be accessed through direct online links to the files from the search results (18, 19, 25). No other sources were used to obtain the selected articles.

Search for common biomarkers identified in primary studies of T2DM in the database of proteomic biomarkers for PCOS

All proteomic biomarkers for T2DM identified in this systematic review from the primary studies were searched on the updated database of proteomic biomarkers for PCOS. Where there were any ‘hits’, a note was made of the original tissue in women with PCOS where these biomarkers had been identified and a note made of their function. This process was independently validated by three of the authors (N G, T A and M X).

Results

Proteomic studies for T2DM

Figure 2 demonstrates the selection process of the primary studies where proteomic methodologies were used for the identification of biomarkers in T2DM. The

Table 1 The main characteristics of each study with the proteins affected in patients with OC compared with normal individuals.

Study	Study design	n	Population		Selection criteria		Proteins identified/increased (+) or decreased (-)	Sample site	Technique used
			Mean age±s.d. and age range (years)	Inclusion	Exclusion				
(30)	Cross-sectional study	Total: 8, control: 4, diabetics: 4	55–75	Edentulous patients, clinical diagnosis of T2DM	Age < 45 years, chronic disease with oral manifestations, gross oral pathology, overt denture abrasion, clinically significant organic diseases, e.g. impaired renal function, bleeding disorder, active infectious diseases immunosuppression due to medications/conditions; using any antibiotic or antifungal for any medical 1 month pre-screening; using ongoing medications initiated < 3 months pre-enrolment; known or suspected intolerance to local oral anaesthesia; having participated in another clinical study; used tobacco products 6 months pre-screening; previously participated in this study; post-menopausal women on HRT	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (+), serum amyloid A protein (SAA) (+), cystatin S (CYTS) (+), cystatin SN (CYTN) (-), prolactin-inducible protein (PIP) (+), carbonic anhydrase 6 (CAH 6), uteroglobin (UTER) (+), protein PLUNC (-), amylose 2B (AMY 2B) (-), serotransferrin (TRFE) (-), alpha-amylose 1 (AMY1) (-), alpha-2-macroglobulin (A2MG) (-)	Saliva	2D-LC-MS/MS	
(31)	Case-control study	Total: 24, lean non-diabetic, controls: 8, obese non-diabetic controls: 8, type 2 diabetics: 8 (in all three groups, the female: male ratio was 1:1)	Lean: 37±4, obese: 44±3, diabetics: 48±3	Relevant medical history, physical examination, screening laboratory tests and a 75 g OGTT and underwent an euglycaemic mic-hyperinsulinaemic clamp with basal muscle biopsies	Not stated	Cytochrome c oxidase polypeptide VIC precursor (-), coiled-coil-helix-coiled-coil-helix domain-containing protein 3 (-), ubiquinol-cytochrome c reductase complex ubiquinone-binding protein QP-C (-), desmin; mutant desmin (-), alpha actinin-2 (-), myozenin-1 (-), cullin homologue 5 (+), proteasome subunit beta type 3 (+), myosin-15 (+), HSP90 co-chaperone CDC37 (-), T-complex protein 1 subunit delta (+), chaperonin containing TCP1, subunit 8 (theta) variant; T-complex protein 1 subunit theta (+), glutamyl-TRNA synthetase (+), 55 kDa protein; protein disulfide-isomerase A3 precursor (+), NG,NG-dimethylarginine dimethylamino-hydrolase 1 (+)	Saliva	2D-LC-MS/MS and pathway analyses including western blotting	
(32)	Cross-sectional study	Total: 20, controls: 12, type 2, diabetics: 8	48–65	Not available	Not available	Flotillin-1 (+), syntaxin 1C (-), arignase (+)	Serum	Electrophoresis (1-dimensional and 2-dimensional), digital image analysis, MALDI-TOF MS, western blotting, SDS-PAGE	

Table 1 Continued

Study	Study design	n	Population		Selection criteria		Proteins identified/increased (+) or decreased (-)	Sample site	Technique used
			Mean age ± s.d. and age range (years)	Inclusion	Exclusion				
(33)	Case-control study	Total: 10 adult male subjects, non-diabetic controls: 5, diabetics: 5	Non-diabetic subjects: 67.6 ± 1.67, type 2 diabetic patients: 67 ± 1.71	All subjects older than 65 years	No diabetes history in control subjects	ALB protein (+), complement component C4B (+), complement component 4A (+), complement C4 precursor (+), alpha-2-macroglobulin precursor (+), alpha 2 macroglobulin variant (+), complement factor H (+), C4B1 (+), complement component 4B preproprotein (+), Ig mu heavy chain disease protein (+), complement C3 precursor (+), immunoglobulin heavy constant mu (IGHM) (+), immunoglobulin heavy constant mu (IGHM) (+), afamin precursor (+), 65 kDa protein (+), hemopexin precursor (+), C4b-binding protein alpha chain precursor (+), hypothetical protein DKFZp686C15213 (+), haptoglobin precursor (+), alpha-1-antitrypsin precursor (+), splice isoform 1 of complement factor B precursor (+), apolipoprotein E precursor (+), serum amyloid A-4 protein precursor (+), apolipoprotein C-III precursor (+), complement C1q subcomponent, A chain precursor (+), apolipoprotein A-I precursor (+), casein kinase II beta subunit (+), alpha 2 globulin variant (+), the human immunoglobulin heavy diversity (IGHD) (+), keratin, type I cytoskeletal 19 (+), hypothetical protein DKFZp686I04196 (+), quiescin Q6 (+), splice isoform 1 of C-reactive protein precursor (+), keratin, type I cytoskeletal 12 (+), keratin, type I cytoskeletal 15 (+), keratin, type I cytoskeletal 16 (+), the human immunoglobulin heavy diversity (IGHD) (+), hypothetical protein DKFZp686C02220 (+), keratin, type I cytoskeletal 17 (+), keratin, type I cytoskeletal 13 (+), complement component C8 gamma chain precursor (+), ficollin-3 protein (+), hypothetical protein (+), amyloid lambda 6 light chain variable region SAR (+), ceruloplasmin precursor (+), VH4 heavy chain variable region precursor (+), hypothetical protein DKFZp686E23209 (+), ficollin-2 precursor (+), apolipoprotein A-II precursor (+), hypothetical protein FLJ20261 (+), complement component C8 alpha chain precursor (+), hepatocellular carcinoma-associated protein TB6 (+), keratin, type I cytoskeletal 14 (+), apolipoprotein C-II precursor (+), S100 calcium-binding protein A7 (+), hypothetical protein FLJ41805 (+), 115 kDa protein (+), platelet factor 4 precursor (+), keratin, type II cytoskeletal 6A (+), Fc fragment of IgG binding protein (+), myosin-reactive immunoglobulin kappa chain variable region (+), keratin, type II cytoskeletal 6B (+), keratin, type II cytoskeletal 6E (+), serum amyloid A protein precursor (+), keratin, type II cytoskeletal 2 epidermal (+)	Serum	MA-plotting analyses, SDS-PAGE, LC-MS/MS analysis, localized statistics of protein abundance distribution (LSPAD)	

Table 1 Continued

Study design	Study n	Population		Selection criteria		Proteins identified/increased (+) or decreased (-)	Sample site	Technique used
		Mean age ± s.d. and age range (years)	Inclusion	Exclusion				
(34) Cross-sectional study	Total: 15, T2DM: 5 (one male, four females), controls without history of diabetes: 10 (five females, five males)	Mean age ± s.d. and age range (years) T2DM: 66 ± 6 controls: 62 ± 6	Control and diabetic samples were from age- and weight-matched donors, trauma-related deaths in controls	Not available	Insulin (-), prolactin (-), PDGF (-), interferon (IFN) (-), mTOR to S6, eIF4E (-), oncostatin M (-), lipopoly-saccharide (LPS) to c-Jun N-terminal kinase (JNK) (-), T-cell receptor (TCR) (-), B-cell receptor to NF-AT (-), E2F (+), P53 (+), Fas (+), Apo2 Ligand (+), caspase (+), stress-associated protein (+), EGF (+), receptor activator of nuclear factor kappa ligand (RANKL) (+), Parkin associated (+), polo-like kinase 1 (PLK1) (+), toll-like receptor 3 (TLR3) (+)	Cadaveric pancreatic islet cells	SELDI-TOF MS, liquid chromatography Fourier transform ion cyclotron resonance mass spectrometry (LC FT-ICR MS)	
(35) Case-control study	Total: 40, controls: 10 (age 36-62 years), diabetes: 10, IGT: 10, IGT + IFG: 10	Controls: 46.2 ± 11.32, IGT: 43.8 ± 13.18, IGT + IFG: 48.8 ± 8.23, T2D: 49.75 ± 7.89	IFG (impaired fasting glucose) if the fasting plasma glucose level was elevated (between 100 and 125 mg/dl after an overnight fast) and IGT (impaired glucose tolerance) if the 2-h plasma glucose level was elevated (between 140 and 199 mg/dl) after an OGTT oral glucose tolerance test. Diagnosis of diabetes based on American Diabetes Association criteria	For control group: pregnancy, alcohol consumption, tobacco products (former or current), chronic medical illness and history of any drug treatment within the previous 3 months. Gingival inflammation, other oral disease	Carbonic anhydrase 6 (+), pyruvate kinase isozymes M1/M2 (+), glycogen phosphorylase, liver form (+), transferrin (+), neutrophil collagenase (+), purine nucleoside phosphorylase (-), transmembrane protease, serine 11D (-), aldehyde dehydrogenase, dimeric NADP-prefering (-), isoform 2, 3 and 4 of chitotriosidase-1 (+), cathepsin Z (-), phosphoglycerate kinase 1 (-), aldo-keto reductase family 1 member B10 (-), apolipoprotein B-100 (-), carbonic anhydrase 1 (-), carbonic anhydrase 2 (-), proteasome (prosome, macropain) subunit, beta type, 7 (-), calnexin (-), uteroglobin (+), serpin peptidase inhibitor, clade B (+), protein Plunc (+), complement component C6 (+), alpha-1-antitrypsin (+), cystatin-C (+), leukocyte elastase inhibitor (+), cystatin-A (-), annexin A1 (-), histone cluster 1, H1e (+), neuroblast differentiation-associated protein AHNAK (+), calmodulin-like protein 5 (-), fatty acid-binding protein, epidermal (-), peroxiredoxin-1, -2 and -6 (-), small proline-rich protein 2F (-), protein S100-A7 (-), lamin A/C (-), annexin A2 (-), desmoplakin (-), flavin reductase (-), ribonuclease pancreatic (+), interalpha-trypsin inhibitor heavy chain H1 (+), chitinase-3-like protein 1 (+), interalpha-trypsin inhibitor heavy chain H4 (+), nucleobindin-2 (+), kallikrein-13 (-), kallikrein-10 (-), basic proline-rich peptide IB-8a (+), acidic leucine-rich nuclear phosphoprotein 32 family (+), retinol binding protein 4, plasma (+), cofillin-1 (+), 14-3-3 protein epsilon (-), annexin A3 (+), 14-3-3 protein eta (-), actin-related protein 2/3 complex subunit 5 (+), isoform 2 of P60953 cell division control protein 42 homologue precursor (+), alpha-2-macroglobulin (+), grancalcin (-), actin-like protein (+), P26038 moesin (+), Ly6/PLAUR domain-containing protein 3 (-), isoform 2 of P67936 tropomyosin alpha-4 chain (-)	Saliva	2D-LC-MS/MS, 2-DLC, western immunoblotting, ELISA	

Table 1 Continued

Study	Study design	n	Population		Selection criteria		Proteins identified/increased (+) or decreased (-)	Sample site	Technique used
			Mean age \pm s.d. and age range (years)	Inclusion	Exclusion				
(36)	Case-control study	Total: 175, diabetics: 125, controls: 50	35–65	American Diabetes Association for type 2 diabetes criteria between 12 October 2006 and 1 December 2006	American Diabetes Association for type 2 diabetes criteria between 12 October 2006 and 1 December 2006		C-reactive protein (-), apolipoprotein A-1 (-), apolipoprotein E (+), leptin (+)	Serum	2-DLC, chromatofocusing, RP-HPLC, MALDI-TOF, ELISA
(37)	Case-control study	Total: 6, diabetics: 3, controls: 3	54–65	After a 75 g OGTT, the study participants were categorized using the WHO criteria (1999) as having NGT (normal glucose tolerance) or T2DM, normal blood pressure, non-smoking, normal thyroid, liver, cardiopulmonary and kidney function as determined by medical history, physical examination and blood chemistry screening	Other metabolic or cancerous disease, medication affecting insulin secretion		Apolipoprotein C3 (+), transthyretin (+), albumin (-), transferrin (-)	Serum	SELDI-TOF MS, fractionation, SDS-PAGE, PMF
(38)	Case-control study	Not specified	Not specified	Sera samples were obtained from volunteers at St Catherine's and St Joseph's Hospital in Hamilton, Sunnybrook Hospital in Toronto and London Health Science Center in London, Ontario, Canada. Some of the normal samples were obtained from Intergen (Massachusetts, USA)			Pro-platelet basic protein (+), serine (or cysteine) proteinase inhibitor, clade A (-), serine (or cysteine) proteinase inhibitor, clade C (-), vitronectin precursor (+), alpha-2 macroglobulin (+), complement C4 (+), fibronectin (+), C1 inhibitor (+), alpha 1B-glycoprotein (+), alpha-1-antitrypsin (-), apolipoprotein AI (-), apolipoprotein B100 (+), complement component 3 (+), factor H (-), haptoglobin alpha 2 chain (+), haemoglobin alpha chain (+)	Serum	Multidimensional liquid chromatography, Gel electrophoresis coupled to matrix-assisted laser desorption/ionization quadrupole time-of-flight, ion trap liquid chromatography-tandem mass spectrometry, western blot analysis, ELISA

n, number of participants; OGTT, oral glucose tolerance test; MALDI-Q-TOF-MS, matrix-assisted laser desorption/ionization quadrupole time-of-flight MS; MS, mass spectrometry; 2DE, 2D gel electrophoresis; 2D-LC-MS/MS, multidimensional liquid chromatography/tandem mass spectrometry; 2-DLC, two-dimensional liquid chromatography; SELDI-TOF MS, surface-enhanced laser desorption/ionization time-of-flight mass spectrometry; PMF, peptide mass fingerprinting; RP-HPLC, reverse-phase high-performance liquid chromatography; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

1. Description of selection criteria.
2. The spectrum of patients used in each study is representative of the patients who will receive the test in practice.
3. Full description of the sample size.
4. Adequate description of the procedure and timing of the collection of biological sample with respect to clinical factors.
5. Adequate description of handling and pre-analytical procedures-were these the same for the whole sample?
6. The period between the reference standard and the index test is short enough to reasonably guarantee that the target condition did not change between the two tests.
7. The reference standard is likely to correctly classify the target condition.
8. The whole sample or a random selection of the sample received verification using a reference standard of diagnosis.
9. The patients received the same reference standard regardless of the result of the index test.
10. The execution of the index test is sufficiently described to its permit replication.
11. The execution of the reference standard is sufficiently described to its permit replication.
12. The index test results are interpreted without knowledge of the results of the reference standard.
13. The reference standard results are interpreted without knowledge of the results of the index test.
14. The same clinical data is available when test results are interpreted as it would be when the test is used in practice.
15. Any uninterpretable/ intermediate test results are reported.
16. The presence of overfitting was most likely avoided.

Figure 1 According to QUADOMICS Tool, the following methodological criteria were applied to this review.

initial search conducted through MEDLINE yielded 145 articles that included 30 reviews. After screening the titles and abstracts, 17 primary studies were isolated. Studies were excluded if they were review articles, proteomic techniques were not used or if they did not compare T2DM with a healthy control group. Six studies involving animals only and two comparing different proteomic approaches were further excluded leaving nine primary studies (30, 31, 32, 33, 34, 35, 36, 37, 38) evaluated in this review. Further searches of the Cochrane and EMBASE databases and hand searching of the references of relevant manuscripts did not yield additional articles.

General characteristics of the studies included

A total of nine studies were identified from the literature (Table 1) involving at least 290 participants (one study did not specify its sample size (38)). In the studies, the samples were collected from different sites: serum in five studies (32, 33, 36, 37, 38), saliva in three studies (30, 31, 35) and Cadaveric pancreatic islet cells in one study (34). With the exception of one study, the selection criteria were overall adequately described (32). The study population was fully described in eight studies whereas Zhang *et al.* (2004) (38) failed to state its sample size. Various proteomic techniques were used in the nine studies with surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF), matrix-assisted laser desorption time-of-flight (MALDI-TOF) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) being the most common (Table 1).

Assessing the quality of the relevant studies

Table 2 summarizes the quality assessment process in accordance with the QUADOMICS Tool (17). Six out of the nine studies were classified as HQ fulfilling at least ten out of the 16 criteria (30, 31, 33, 34, 35, 37). The three remaining studies were classified as LQ achieving 8 or 9 out of the 16 quality (32, 36, 38). Of particular note was that none of the studies explicitly stated that the index test results were interpreted without knowledge of the results of the reference standard and vice versa, thus failing to achieve the 12th and 13th criteria of the QUADOMICS (17). Furthermore, the 2nd and 14th quality criteria of the QUADOMICS (Fig. 1) were not applicable (NA) for this review as the studies were conducted on T2DM patients of mixed sex and older age compared with the PCOS patients that are the subject of interest of our study.

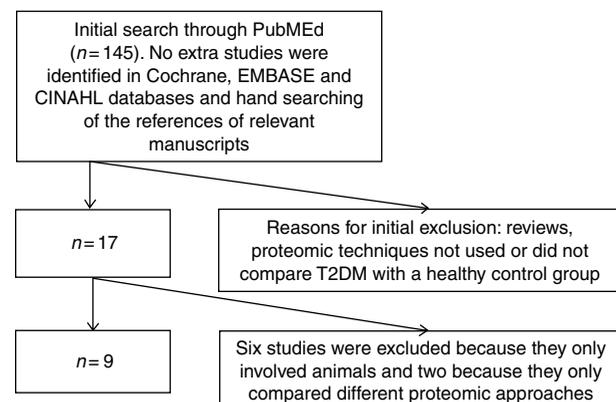


Figure 2 A flow chart summarizing the selection process.

Table 2 Methodological Quality Assessment using the QUADOMICS Tool (17).

Quality criteria	(30)	(31)	(32)	(33)	(34)	(35)	(36)	(37)	(38)
1	Y	Y	Y	Y	Y	Y	N	Y	N
2	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	Y	Y	N	Y	Y	Y	N	Y	N
4	Y	Y	Y	Y	Y	Y	Y	Y	Y
5	Y	Y	Y	Y	Y	Y	Y	Y	Y
6	Y	Y	Y	Y	Y	Y	Y	Y	Y
7	Y	Y	Y	Y	Y	Y	Y	Y	Y
8	Y	Y	Y	Y	Y	Y	Y	Y	Y
9	Y	Y	Y	Y	Y	Y	Y	Y	Y
10	Y	Y	Y	Y	Y	Y	Y	Y	Y
11	Y	Y	Y	Y	Y	Y	Y	Y	Y
12	N	N	N	N	N	N	N	N	N
13	N	N	N	N	N	N	N	N	N
14	NA	NA	NA	NA	NA	NA	NA	NA	NA
15	N	N	N	N	N	N	N	N	N
16	N	Y	N	Y	N	Y	Y	N	N
Total	10/16, HQ	11/16, HQ	9/16, LQ	11/16, HQ	10/16, HQ	11/16, HQ	9/16, LQ	10/16, HQ	8/16, LQ

Quality criteria numbers 1-16 are described in Fig. 1. Y, criterion achieved; N, criterion not achieved or not mentioned; HQ, high quality; LQ, low quality; NA, not applicable.

Cross-referencing proteomic biomarkers identified in primary studies of T2DM in the database of proteomic biomarkers for PCOS

Thirty-two additional proteomic biomarkers for PCOS were identified in the process of updating the PCOS proteomic database (available on request) and these were merged with the old database (16). Some biomarkers were variants of the same protein that was presumed to be due to varied post-translational modifications or splicing variants. A free text search of the PCOS proteomic biomarker database was carried out using the 191 T2DM proteomic biomarkers identified in the nine studies evaluated in the systematic review of T2DM proteomic biomarkers.

This exercise identified eight proteomic biomarkers that were differentially expressed (either over- or under-expressed) in both patients with PCOS and with T2DM when compared with healthy controls. These biomarkers include pyruvate kinase M1/M2 (PKM1/M2), apolipoprotein A-I, albumin, peroxiredoxin 2, annexin A2, α -1-B-glycoprotein (A1BG), flotillin-1 and haptoglobin.

Discussion

This study has, for the first time, identified a panel of eight proteomic biomarkers that were differentially expressed (either over- or under-expressed) in both women with PCOS and women with T2DM when compared with healthy controls. These are interesting findings but they will need to be validated, and further studies will need to investigate how these proteins interact with themselves and other proteins to explain the link between PCOS and T2DM. A consistently emerging theme from studies using proteomic

approaches in PCOS is the potential role of immunoregulation/inflammation and antioxidants in the pathogenesis of the condition. These two pathways have also been implicated in T2DM and insulin resistance, both of which are of concern in women with PCOS (1, 2, 3, 4, 5, 6, 7).

Our findings show PKM1/M2 to be elevated both in patients with PCOS and with T2DM. Pyruvate kinase catalyzes the last step of glycolysis where phosphoenolpyruvate is converted to ADP. PKM2 is known to interact with a variety of biological molecules such as A-Raf, FGFR-1 and Jak-2 mutant and is also implicated in cancer metabolism (39), which could contribute to the increased incidence of certain malignancies observed in T2DM and PCOS compared with the rest of the population (40, 41). For this reason, it should be further explored whether the increased levels of PKM1/M2 observed in both T2DM and PCOS may represent a common defect in glucose metabolism.

Apolipoprotein A-I was found to be down-regulated in both PCOS and T2DM when compared with healthy controls. This is the transporter protein of HDL and its down-regulation in both T2DM and PCOS is associated with the reduction of HDL levels (in combination with the proposed increase in fractional HDL clearance especially in T2DM) (42). The reduced levels of ApoA-I present in the granulosa cells of women with PCOS could affect cholesteryl ester uptake, which, in turn, would be expected to disturb normal steroidogenesis pathways by limiting the initial substrate (18). Both these mechanisms could contribute to the increase in prevalence of cardiovascular and cerebrovascular accidents observed in patients with T2DM and PCOS compared with the rest of the population. As a result, the presence of down-regulated apolipoprotein A-I in women with PCOS could be associated with the development of T2DM and/or cardiovascular complications.

As supported by the Deckert's Steno hypothesis, urinary albumin excretion, a marker of an atherogenic milieu, directly reflects the state of endothelial function and early endothelial damage (43). Our findings show albumin to be down-regulated in both patients with T2DM and PCOS. Many aetiologies have been proposed in the case of T2DM including the fact that poorly controlled diabetic patients may have elevated vascular and renal permeability to albumin and hence decreased serum albumin (44). Similarly, the reduced albumin levels in women with PCOS can be attributed to the subclinical atherosclerotic changes evident in women with PCOS (7, 8, 9). Given the fact that albumin levels can be determined with relatively simple tests, albumin could practically be an important biomarker in determining the risk of developing T2DM and/or cardiovascular disease in women with PCOS.

Peroxiredoxin 2 was another protein found to be down-regulated in both patients with T2DM and PCOS. Peroxiredoxin-2 has an antioxidant activity and its down-regulation in both PCOS and T2DM indicates oxidative stress and toxicity processes (45). In fact, oxidative stress has been suggested to occur in many disorders related to insulin resistance like T2DM and PCOS (46). Furthermore, studies have recently suggested that reactive oxygen species induce oxidative stress that stimulates androgen-producing steroidogenic enzymes leading to the hyperandrogenism observed in women with PCOS (47, 48).

Our review also found that annexin A2 was down-regulated in both patients with T2DM and PCOS. Annexin A2 is the main physiological receptor for plasminogen on the extracellular surface of endothelial cells and has been found to be a vulnerable target for glycation, quickly responding to restoration of normoglycaemia (49). Its glycation may impair the appropriate formation of the plasminogen/tissue plasminogen activator/annexin complex, disrupting a key regulatory mechanism in fibrinolytic vigilance. It is thus plausible to postulate that, in T2DM, the delicate balance of coagulation/fibrinolysis would be tilted towards enhanced coagulation and thrombosis (49). We thus hypothesize that the down-regulation of annexin A2 found in some women with PCOS could be a strong marker for the development of T2DM in this group.

Our exercise found that A1BG was over-expressed in both patients with T2DM and PCOS. A1BG was discovered decades ago; however, no biological function or association to any disease has been assigned to it yet (50). A study found that A1BG was higher in premenopausal women when compared with men of the same age (51). These findings are consistent with other results where aging and sex hormones were suggested to contribute to the expression of A1BG (50). Another study suggested that A1BG is a member of the immunoglobulin family, which serves diverse functions based on molecular recognition especially in the immune system and in cell adhesion (52). Thus, the

hormonal and immunological disturbances in both PCOS and T2DM might explain this over-expression.

Our exercise also found that flotillin-1 was over-expressed in both patients with T2DM and PCOS. Flotillin-1 stimulates the activation of glucose transporter 4 in response to insulin and is a regulator of insulin function (32). It is thus over-expressed in the hyperinsulinaemic state of T2DM. It is established that PCOS is associated with hyperinsulinaemia, insulin resistance, abnormal glucose metabolism and ultimately T2DM (53); therefore, the presence of flotillin-1 in PCOS women may predict the development of T2DM.

The final protein identified to be over-expressed in both T2DM and PCOS was haptoglobin. This protein interacts with haemoglobin and is found to be elevated in response to inflammation and oxidative stress (38). Its over-expression in both PCOS and T2DM could thus be explained on the basis that both conditions have an inflammatory component (3, 4, 54).

Although proteomic and other 'omic' technologies offer a great potential for the generation of new insights into disease aetiology, concerns have been expressed about the relatively slow pace at which research findings have been translated into clinical care (55). There has been a call for greater focus on data integration from primary proteomic studies in order to improve translation of research findings and prospective validation (56). The sample sizes and number of biomarkers identified following these studies runs the risk of false positives and this is a limitation of all biomarker studies. These issues again emphasize the need for collaboration, data synthesis and integration (as done in this review) in order to identify a shortlist of replicated biomarkers that can be validated in subsequent hypothesis-driven research. We therefore see great value in informing the scientific community about these research findings at this stage as in the area of 'omic' research, data sharing and collaboration is vital for progress. For example, an independent research group with access to stored tissue samples from women with PCOS who have had T2DM may, based on this review, decide to independently verify the biomarkers identified in their cohort that would save time.

In summary, by integrating data from proteomic studies in T2DM with data from proteomic studies in PCOS, we have for the first time identified a panel of eight promising biomarkers of T2DM in women with PCOS including PKM1/M2, apolipoprotein A-I, albumin, peroxiredoxin 2, annexin A2, A1BG, flotillin-1 and haptoglobin. If validated, these biomarkers could provide a useful framework on which the knowledge base in this area could be developed and will facilitate future mathematical modelling to enhance screening and prevention of T2DM in women with PCOS who have been shown to be at increased risk. A well-coordinated multidisciplinary collaboration of basic scientists, clinicians and mathematicians is vital to achieve this goal.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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A systematic review of the literature identifying eight potential proteomic biomarkers expressed in both patients with PCOS and T2DM.

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