**Literature-based candidate protein selection**

**PubMed NCBI literature search (Aug. 2013); total hits = 731**

- "GDM biomarker srn (0)", "GDM biomarker mrm (0)", "GDM biomarker (103)", "GDM protein biomarker (69)", "GDM proteomics (2)", "Gestational diabetes proteomics (3)", "gestational diabetes proteomic (6)", "gestational diabetes mass spectrometry (34)", "gestational diabetes biomarker (223)", "srn biomarker" (135), "mrm biomarker" (156) - *evaluation based on title content*

**Candidate protein selection:**

*Include:* GDM markers, T2DM markers ≥ 2 studies, markers associated with opposing results, markers described in GDM reviews  

*Exclude:* Proteins related to coagulation, data based on enzyme activity

- **54** candidate proteins of interest selected (listed below)

- **47** candidate proteins selected for MRM assay development based on method compatibility e.g. unique unmodified tryptic peptides (m/z<1500) and reported serum abundance (for details see MRM-MS assay development section above)

- **25-plex MRM assay** developed and method validated

- 2 candidate proteins (medical records)
- 2 candidate proteins (ELISA)

**Serum analysis, data extraction and statistical analysis**
## 54 GDM biomarker candidates

1. Adiponectin $Q15848$
2. AFP $P02771$
3. Alpha-1-antichymotrypsin (SERPIN-A3) $P01011$
4. Alpha-2-4HS-glycoprotein-Fetuin-A (AHSG) $P02785$
5. Alpha-2-macroglobulin $P01221$
6. Angiopoietin-related protein 2 (ANGPTL2) $Q6UK19$
7. Angiopoietin-related protein 6 $Q8N199$
8. Apelin $Q9ULZ1$
10. Apolipoprotein A-II (Apo-II) $P06552$
11. Apolipoprotein A-IV (Apo-L) $P06727$
12. Apolipoprotein B-100 (Apo-B100) $P04114$
13. Apolipoprotein C-II (Apo-CII) $P02655$
15. Apolipoprotein D (Apo-D) $P05590$
17. Apolipoprotein L1 (Apo-L1) $Q14791$
18. Apolipoprotein M (Apo-M) $Q95445$
19. Apolipoprotein(a) (Apo(a)) $P08519$
21. Beta-thbg $P01233$
22. Ceruloplasmin $P04450$
23. Clusterin beta chain $P10989$ + Clusterin alpha chain $P10999$
24. C-reactive protein (CRP) $P02741$
25. C-X-C motif chemokine 5 (CXCL5) $P42838$
26. Dipeptidyl peptidase 4 (DPP-IV) $P21487$
27. Fetuin light chain $P02792$ + Fetuin heavy chain $P02794$
28. Fibronectin $P02751$
29. Filamin A $P13142$
30. Haptoglobin chain beta $P00738$
31. Insulin-like growth factor 1 (IGF) $P09019$
32. Interleukin-1 beta (IL-1beta) $P01544$
33. Interleukin-18 (IL-18) $Q14116$
34. Interleukin-6 (IL-6) $P05231$
35. Leptin $P41159$
36. MCP-1 $P13500$
37. Nesfatin 1 $P80393$
38. Neutrophil gelatinase-associated lipocalin (NGAL) $P00188$
39. Nicotinamide phosphoribosyltransferase (Vasistrin NAMPT) $P43490$
40. Omentin (TLN-1) $Q8W5O$
41. Osteopontin (OPN/BSP) $P10451$
42. PAPP-A $Q12319$
43. Plasminogen activator inhibitor 1 (PAI-1) $P05121$
44. Resistin $Q9US99$
45. Retinoic acid receptor responder protein 2 (RARRES2) $Q99669$
46. Retinoic-binding protein 4 (RBP-4) $P02753$
47. Secreted frizzled-related protein 5 (SFRP5) $Q5T4F7$
48. Serum amyloid a (SAA1) $Q00207$
49. Sex hormone-binding globulin $P04178$
50. Transferin (Serotransferrin) $P02787$
51. Transthyretin (TTR) $P02766$
52. Tumor necrosis factor (TNF-zeta) $P01375$
53. Vaspin (Serpin A12) $Q4W75$
54. Vitamin D-binding protein (Galectin) $P02774$

Protein marked in green were assayed by MRM-MS and proteins marked in red were assayed by immunological methods. Underlined proteins were validated by immunological methods.