Concept sheet

Title	Multi-molecular biomarker panel for the early screening, molecular distinction and clinical follow-up of patients with various subtypes of preeclampsia
Acronym	PreeclamChip
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Topic	Multi-marker clinical test for preeclampsia
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The concept

The objective of this project is to develop a multi-marker clinical test for the early screening, molecular distinction and clinical follow-up of patients with various subtypes of preeclampsia. The systems biological approach of the authors has recently led to the discovery of a biomarker panel, which – if developed and transformed into a multiplex assay first in the market - may detect preeclampsia and/or HELLP syndrome-specific changes in maternal blood in early pregnancy (<10 weeks of gestation) with high sensitivity and specificity. Based on the proteomics and transcriptomics data, this biomarker panel may also differentiate between the various subtypes of preeclampsia at this early stage of pregnancy, and may also be of help in the follow-up and correct clinical diagnosis of patients, as well as their targeted therapies.

The need

Preeclampsia is a syndrome defined by pregnancy-induced hypertension and proteinuria, which can lead to eclampsia (convulsions), and other serious maternal complications (in 18-20% of the cases) and/or fetal complications (in 40-50% of the cases). Preeclampsia affects approximately 8 million (2-7%) pregnant women in developed countries in every year, but can occur three times as often in certain geographic areas among particular ethnic or social groups. Preeclampsia is a major cause of maternal (in 10-15%) and perinatal (in 10%) mortality. Furthermore, women with preeclampsia will have an 8-fold higher risk of cardiovascular death later in their life, and offsprings born from pregnancies affected by preeclampsia have an increased risk of metabolic and cardiovascular disease and mortality later in their life. HELLP (Haemolysis, Elevated Liver enzymes and Low Platelets) syndrome is an even more severe variant of preeclampsia, which is associated with very high maternal and fetal mortality. As only the short-term complications of preeclampsia and HELLP syndrome cause approximately $7-10 billion in healthcare costs in the USA annually, the early diagnosis, prediction and / or prevention of these syndromes would have a very significant social and

economic impact.

The currently available immunodiagnostical and ultrasound methods are not sensitive or specific enough for the early prediction, detection and distinction between the different subtypes of preeclampsia and HELLP syndrome. Several attempts have been carried out in the past few years to develop and validate biomarkers for these syndromes; however, their results were not satisfactory. The reason for this is that the early diagnosis of syndromes with a heterogeneous molecular background cannot be achieved with the utilization of only one or two biomarkers. For example, the use of angiogenic/anti-angiogenic factors and their combination (e.g. placenta growth factor, soluble Flt-1, soluble endoglin) turned out to be promising for the prediction of early-onset preeclampsia; however, these biomarker molecules are not so valuable in the early prediction of late-onset preeclampsia, which comprise the majority of the cases. Moreover, the imbalance of these molecules is characteristic for the final stage of preeclampsia, but not for the very early events that impairs placentation in these syndromes. Furthermore, these angiogenic/anti-angiogenic factors are not specific biomarkers for preeclampsia in the sense that their imbalance is also characteristic for other obstetrical syndromes (pregnancies complicated with small for gestational age neonates, intrauterine growth restriction, intrauterine fetal death, etc.), as well.

Therefore, more sensitive and specific methodologies are necessary, which utilize multiplex assays comprising several biomarkers that enable the early diagnosis and differentiation 1) between the various subtypes of preeclampsia and HELLP syndrome, and 2) between preeclampsia and other obstetrical syndromes. Such multiplex assays that enable the early prediction, diagnosis and prevention of the severe maternal and fetal complications of preeclampsia and HELLP syndrome currently does not exist on the market, but would attract a huge interest from healthcare providers, and would have a large impact on patient care, pregnancy outcomes in affected women, as well as short and long term health outcomes of affected women and their offspring(s).

The solution

The multidisciplinary, systems biology approach of the authors within an international collaboration led to the discovery of a unique biomarker panel for the various subtypes of preeclampsia, which are repeatedly detectable in maternal blood at 7-9th weeks of gestation. The technical excellence of this collaborative work is based on the inclusion of different high-dimensional biology techniques.

Further R&D efforts in the industry may enable the development of this biomarker panel into a multiplexed assay, which would enable the simultaneous detection of these biomarkers in maternal blood. This multiplexed assay could be used in research laboratories, clinical laboratories and as a point-of-care test for the early and very sensitive prediction and clinical follow-up of patients with preeclampsia and HELLP syndrome. This test would enable the 1) early screening, diagnosis and molecular distinction between late-onset and early-onset preeclampsia, from which the latter is frequently associated with fetal growth restriction and more serious maternal and fetal consequences; 2) the early distinction between preeclampsia and other obstetrical syndromes; and 3) follow-up of patients regard their clinical status and effectiveness of potential therapies.

State-of-the-Art

Currently, singleplex immunoassays are routinely used for the detection of biomarker molecules in preeclampsia and in other obstetrical syndromes. These immunoassays still constitute the gold standard of detection of single proteins in complex biological specimens (in our case in maternal

serum or plasma) because they may allow the sensitive and specific detection of these molecules. Several attempts have been carried out for the combined, simultaneous measurements of certain biomarkers to increase the sensitivity and specificity of the predictive tests for preeclampsia; however, these were mainly limited to the implementation of the measurements of angiogenic and anti-angiogenic biomarkers with singleplex assays. However, increasing body of evidence from experiments employing tools of high dimensional biology (transcriptomics, proteomics, etc.) revealed the molecular heterogeneity of preeclampsia, which suggests the necessity of simultaneous measurements of multiple analytes in small sample volumes of maternal blood in order to screen / diagnose / follow up patients. Currently, there is no established biomarker panel available for the molecular prediction of the various subtypes of preeclampsia, and no automatized clinical immunanalyzer system is available that would enable the simultaneous measurement of this biomarker panel in blood.

Beyond the State-of-the-Art

The genome-wide transcriptomics work by the authors identified genes differentially expressed in the placenta in preeclampsia, which encode for proteins secreted to the maternal serum. These results were confirmed in a large set of independent samples with high-throughput RNA and protein assays. Neural network analysis supported the selection of the best combinations of putative preeclampsia biomarker genes, which combination could also reveal differences in various subforms (early-onset vs. late-onset; complicated with or without small-for-gestational age neonates) of this syndrome. The selected panel of transcriptomic markers had a sensitivity of 91.5% at 75% specificity to differentiate between cases of preeclampsia and controls in a set of 100 placental specimens. Although the differential regulation of these genes is pregnancy-specific, their expression changes may not entirely be specific for preeclampsia.

The proteomics work of the authors identified novel maternal serum biomarkers which are not specific for pregnancy; however, they can distinguish between early-onset preeclampsia associated with intrauterine growth restriction and late-onset preeclampsia without growth restriction, as well as other syndromes.

The combination of these transcriptomic and proteomic biomarkers resulted in the assembly of a panel of molecules, which will enable the detection of preeclampsia-specific changes in maternal blood, and can differentiate between the different subtypes of preeclampsia and other obstetrical syndromes.

The innovation beyond the discovery of this biomarker panel requires further R&D efforts and the development of a novel multiplexed assay, which would enable the simultaneous detection of these molecules in a small amount of maternal blood. This multiplex assay could potentially be used in research laboratories, clinical laboratories and as a point-of-care test. The simultaneous, time-saving and cost-effective measurement of this biomarker panel would provide huge potential for research and laboratory diagnostics in preeclampsia. Furthermore, this multi-marker strategy could be translated into robust diagnostic algorithms and effective population screening.

Why now?

Recent developments in microfluidics, microarray- and microbead technologies, as well as in bioinformatics enable the development of high-throughput immunoassays for biomarker panels. The high-dimensional biology studies of the authors have recently identified a biomarker panel that would enable the early detection and differentiation between the various subtypes of preeclampsia in early pregnancy.