

## Genetics of preeclampsia: paradigm shifts

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**Abstract** Segregation of preeclampsia into early-onset, placental and late-onset, maternal subtypes along with the acknowledgement of the contribution of epigenetics in placentally expressed genes proved to be a key first step in the identification of essential gene variants associated with preeclampsia. Application of this insight to other populations and related pregnancy-induced syndromes, such as HELLP, and acknowledgment of the features shared between chromosomal loci associated with preeclampsia in different populations provide the rationale for new strategies for the identification of susceptibility genes and for new and more effective diagnostic strategies.

Preeclampsia and its variants are considered multifactorial pregnancy-associated diseases that form a spectrum of the same illness (Sibai et al. 2005; Redman and Sargent 2005; Baxter and Weinstein 2004). All models proposed (as outlined recently in two complementary reviews) recognize the importance of the placenta (no placenta, no disease), but differ in the order and rela-

tive contribution of the immunological and vascular events and effects in the mother (Sibai et al. 2005; Redman and Sargent 2005). However, mouse models and progress in the genetics of preeclampsia indicate that different genes segregating with different populations or disease entities initiate at distinct points a common central pathway (Cross 2003; Van Dijk et al. 2005). The central pathway shared in all patients and responsible for the induction of maternal symptoms, as reflected clinically by changes in plasma levels six to ten weeks prior to clinical symptoms, is the PlGF-sFLT1-endoglin axis (Venkatesha et al. 2006; Tjota et al. 2001). Placenta-derived sFLT1 in sera of preeclamptic females can induce hypertension, serum levels correlate with disease severity, and, in combination with soluble endoglin, induces preeclampsia in pregnant rats (Venkatesha et al. 2006). Changed levels of these soluble angiogenic factors, as well as other yet unknown mediators of endothelial activation, produced by the hypoxic placenta induce maternal symptoms by interference with TGF- $\beta$ -mediated NOS-dependent vasodilation (Venkatesha et al. 2006; Soleymanlou et al. 2005; Li et al. 2005). The questions therefore emerge at which points the preeclamptic pathway is initiated genetically and how these genes induce placental dysfunction.

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### Early placental and late maternal preeclampsia

Preeclampsia comes in two forms: early (symptoms < week 34) (type I) and late (symptoms > week 34) (type II) (Table 1) (Redman and Sargent 2005; Egbor et al. 2006; Pijnenborg et al. 2006; Duckit and Harrington 2005; Sklaerven et al. 2005). These forms are different

in genetic risk and inheritance (Duckit and Harrington 2005; Sklaerven et al. 2005). Late-onset, *maternal* preeclampsia arises from the interaction between a *normal* placenta and a predisposed maternal constitution reflecting microvascular disease as occurs with long-term hypertension, diabetes or other states of metabolic dysbalance or reflecting a maternal genetic predisposition due to cis- or transacting genetic variations in interacting genes. Pregnancy in these metabolically or genetically predisposed woman is an increased stress test for life, where the additional demand asked for by the growing fetoplacental unit exceeds the maternal resources. The end of pregnancy, i.e. the onset and process of labour, is a temporary, but strong challenge of the maternal system asked for by the fetoplacental unit in progress of being disconnected from the maternal (vascular) system. In this situation, decompensation near term will present in predisposed women as late peri- or postpartum preeclampsia. Decompensation in this context is meant as the situation where the maternal haemodynamic and metabolic resources fail to comply with and to follow the dynamic and specific changes in the placenta and uterus that precede the onset of labour. Likewise, the biochemical factors disturbed in and informative for this process can be predicted to be multiple, but related to the induction of labour. In contrast, early-onset *placental* preeclampsia starts with *abnormal placentation*, has a high recurrence risk, and runs in families with a clear genetic component (recurrence across generations and occur-

rence within families) (Redman and Sargent 2005; Pijnenborg et al. 2006; Sklaerven et al. 2005). This distinction between early and late preeclampsia not only correlates with the risk factors (Table 1), but also with all animal models of preeclampsia, when growth restriction is used as discriminator (Supplementary Table 1). It should be stressed that, as for early onset preeclampsia, the early form of the HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome is placental in origin. The existence of discordant monozygous twin sisters with only one sister affected confirms that HELLP starts in a gene expressed in the placenta (Thornton and Onwude 1991). The additional, but different genetic contribution of the partner explains why only one twin sister develops disease in discordant monozygous twins.

### A call for consortium style genome-wide association studies

Previous searches for candidate genes (about 50) included genes involved with thrombophilia (*F5*, *F2*), haemodynamics (*AGT*, *REN*, *ACE*), endothelial function (*NOS3*, *EDNI*), cytokines (*TNF*, *IL1A*), oxidative stress (*GSTP1*, *GSTM1*), lipid metabolism (*LPL*), the endocrine system (*ESR1*) and angiogenesis (*VEGF*) (Chappell and Morgan 2006). These approaches have failed to identify a single universally accepted susceptibility gene for preeclampsia. Screening for candidate

**Table 1** Differences between early- and late-onset preeclampsia

	Early-onset	Late-onset
Onset of clinical symptoms	<34 weeks	>34 weeks
Relative frequency (%)	20	80
Risk for adverse outcome	High	Negligible
Association with intrauterine growth retardation	Yes	No
Clear familial component <sup>a</sup>	Yes	No
Placental morphology	Abnormal <sup>b</sup>	Normal <sup>b</sup>
Aetiology	Placental <sup>c</sup>	Maternal <sup>d</sup>
Risk factor (relative risk)	Family history (2.9)	Diabetes (3.56) Multiple pregnancy (2.93) Raised blood pressure at booking (1.38) Raised body mass index (2.47) Maternal age $\geq$ 40 (1.96) Cardiovascular disorders (3.84)

<sup>a</sup> Defined as recurrence across generations and occurrence within families

<sup>b</sup> Egbor et al. (2006) Morphometric placental villous and vascular abnormalities in early- and late-onset pre-eclampsia with and without foetal growth restriction. BJOG 113:580–589

<sup>c</sup> Reduced extravillous trophoblast invasion

<sup>d</sup> Predisposed maternal constitution reflecting microvascular disease or predisposed genetic constitution with cis- or transacting genomic variations subject to interaction

genes with selection based on function, rather than confirmed linkage or association with genome-wide significance, are subject to non-replication and inadequate prioritizing of polymorphisms with different relative risks depending on the SNP selected.

Efforts allowing consortium style, large scale genome-wide association studies permitting convincing statistical evidence ( $P < 10^{-8}$ ) at the genome-wide level are urgently needed. To detect an effect with an odds ratio of 1.5 at a minor allele frequency of 0.1 with 80% power at  $\alpha = 5 \times 10^{-7}$ , the analysis of 2,000 cases and 3,000 controls is needed (Todd 2006). This number is now clearly within reach for preeclampsia. Combination of the initiatives as the Dutch Preeclampsia study (150 affected sibpair families selected from 3,000 patients), the British Genetics of Pre-Eclampsia (GOPEC) consortium (627 index pregnancies), and the Norwegian HUNT cohort (1138 preeclamptic women) complemented with the cohorts from Australia/New Zealand (34 pedigrees), Finland (15 families, 350 index pregnancies) and other countries provide adequate size and therefore power to provide precise genetic risks with narrow confidence intervals (The GOPEC consortium 2005; Lachmeijer et al. 2001; Arngimsson et al. 1999; Moses et al. 2000; Laasanen et al. 2003; Laivuori et al. 2003; Moses et al. 2006; Holmen et al. 2003).

However, several aspects should be taken into account before embarking on this expedition. The affected sibpair selection as done in the Dutch study, besides permitting powerful linkage analysis, had two additional advantages (Van Dijk et al. 2005; Lachmeijer et al. 2001). The preeclamptic females studied were not restricted to primigravids. The majority of affected sibpairs in these families, in addition to having preeclamptic pregnancies themselves, were born from preeclamptic mothers, i.e. from females that had more than one preeclamptic pregnancy. By design, these preeclamptic patients were therefore not limited to first pregnancies. Although the preferential occurrence of preeclampsia in first pregnancies is considered central to the primiparity concept underlying the immune-maladaptation hypothesis (Sibai et al. 2005), our cohort neither supports this nor selects for this. The latter guarantees the absence of confounding factors. Secondly, this selection with inclusion of both parents and two affected sibs targets the early familial form of preeclampsia associated with intrauterine growth retardation, i.e. placental preeclampsia (Van Dijk et al. 2005; Lachmeijer et al. 2001; Oudejans et al. 2004). The GOPEC study and studies with similar design appear biased towards maternal preeclampsia, as indicated by the absence of growth restriction (The GOPEC con-

sortium 2005). Given this difference in the type of preeclampsia studied in both cohorts and provided this distinction is recognized in other cohorts, the Dutch and British cohorts likely target different genes, but are complementary for placental and maternal preeclampsia, respectively.

### Transcription based prioritization of candidate regions and genes

The high incidence of preeclampsia with androgenetic pregnancies, the induction of preeclampsia in knock-out mice mutated for the imprinted, maternally expressed p57kip2 (*CDKN1C*) gene, and the presence of confirmed or predicted imprinted genes in loci associated with preeclampsia led to the exploration of the contribution of epigenetics in the aetiology of preeclampsia (Van Dijk et al. 2005; Oudejans et al. 2004). This approach proved essential in the Dutch preeclampsia study with identification of *STOX1*, coding for a novel member of the winged helix domain containing proteins related to the forkhead (*FOX*) multi-gene family. Following confirmation of linkage with the 10q22 region and the identification of a parent-of-origin effect, candidate genes were prioritized by transcription-based analysis (Van Dijk et al. 2005; Oudejans et al. 2004). Genes were targeted for mutation analysis by selection for genes with downregulated expression in androgenetic placentas. This approach of transcription-based prioritization of candidate genes in regions with linkage was recently taken to a higher level through genome-wide expression screening by the Australian/New Zealand group (Moses et al. 2006). Assuming that perturbed mRNA expression in the decidua is a recognizable signature for maternal preeclampsia, gene expression was quantified using Whole Genome Bioarrays comprising ~55,000 30-mer oligonucleotide probes. Comparison of normalized expression intensity between normotensive and preeclamptic decidua yielded 17 transcripts within the region with linkage (2q22) with at least a 2.5-fold upregulation in association with preeclampsia. Highest priority was given to the activin receptor gene, *ACVR2*. This gene, in addition to a greater than tenfold difference in gene expression between normotensive and preeclamptic decidua, yielded the highest score by GeneSniffer analysis. One SNP (rs1424954) showed preliminary evidence of association with preeclampsia ( $P = 0.007$ ). The gene within the 1-LOD support interval on 2q with the largest increase in relative intensity (>70 fold) encodes the candidate mediator of the p53-dependent G2 arrest (*REPRIMO*). The potential of this approach

has been confirmed recently. In the Norwegian population, three SNPs within the *ACVR2* gene were confirmed to be associated with preeclampsia (Tommerdal et al. 2006). The strongest association was found with rs1424941 ( $P = 0.013$ ). Therefore, the 2q22 region appears to contain a common susceptibility locus associated with *maternal* preeclampsia, which is present in multiple populations (Australia/New Zealand, Iceland, Norway) (Table 2). Restricted association analysis by chromosome-specific, high density SNP analysis might permit identification in a timely and less costly manner of the actual polymorphic variations responsible for preeclampsia in these populations.

### Dichotomous nature of genetic variations

A prevalent mutation consisting of an absolutely conserved Y153H variation in the DNA binding region of the winged helix domain containing *STOX1* gene segregates maternally and is associated with the preeclamptic phenotype in all Dutch families studied (Van Dijk et al. 2005). However, the Y153H variation occurs in the normal population as well, and is in fact the predominant variation in certain populations such as the

CHB population (Han Chinese in Beijing) ([www.hap-map.org](http://www.hap-map.org)). Given the large region on 10q22 with parent of origin effect and the fact that similar variations in related forkhead genes (such as *FOXCI*) cause disease by interaction with downstream genes differentially interacting with wildtype and mutant variants, a second gene variation is needed to explain the full phenotype in Dutch females (Van Dijk et al. 2005). This second gene most likely resides on 10q22 as well. This dichotomous nature of two gene variations in interacting genes appears common rule rather than the exception in preeclampsia. This parallels the recent findings from other common diseases with low effect involving frequently occurring polymorphisms. This has been demonstrated for the two genes (complement factor B, *CBF*; complement component 2, *C2*) associated with age-related macular degeneration and can be expected to be the case for the variant of transcription factor 7-like 2 gene (*TCF7L2*) associated with type 2 diabetes (Gold et al. 2006; Grant et al. 2006). The Australian/New Zealand group corrected for this by applying variance components-based linkage analysis (Moses et al. 2006). Using this approach, data from pedigree structures of arbitrary complexity can be used to make inference regarding the localization and effect sizes of quantitative trait

**Table 2** Loci associated with preeclampsia

Country	Intake	Study design	Test	F/C	Loci	Marker	Gene	<i>P</i>
A. Epigenetic loci associated with placental preeclampsia <sup>a</sup>								
Australia/NZ	1984–1999	Family	VCL <sup>b</sup>	34	2p11.2	D2S388		1.36 <sup>e</sup>
Iceland	1984–1993	Multigenerational	GWS <sup>c</sup>	2	2p13	D2S292-D2S329		3.49 × 10 <sup>-6</sup>
Finland	1994–1999	Case-control	Association	133	2p13	D2S286		0.03 <sup>d</sup>
Netherlands	1995–1997	Affected sibs	GWS	34	10q21.3	D10S1432	STOX1	
B. Locus associated with HELLP								
Netherlands	1995–1997	Affected sibs	GWS	38	12q24	PAH		2.1 <sup>e</sup>
C. Common loci associated with maternal preeclampsia								
Australia/NZ	1984–1999	Family	VCL	34	2q22.3	rs1424954	ACVR2A	0.007
Norway	1995–1997	Case-control	Association	1,138	2q22.3	rs1424941	ACVR2A	0.013
Iceland	1984–1993	Multigenerational	GWS	70	2q23.3	D2S321		2.08 <sup>e</sup>
D. Loci associated with the parahox paralogon								
Australia/NZ	1984–1999	Family	VCL	34	5q15	D5S644		3.12 <sup>e</sup>
Australia/NZ	1984–1999	Family	VCL	34	13q33.3	D13S173		3.10 <sup>e</sup>
Australia/NZ	1984–1999	Family	GWS	15	4q34	D4S450-D4S610		2.9 <sup>e</sup>
Finland	1997–1999	Multigenerational	GWS	15	4q32.1-3	D4S413-3046		0.003238
E. Loci associated with Finnish families								
Finland	1997–1999	Multigenerational	GWS	15	2p25.1	D2S168		0.000761
Finland	1997–1999	Case-control	Association	350	2p25.1	D2S1400		0.00036
Finland	1997–1999	Multigenerational	GWS	15	9p21	D9S169		0.000821

<sup>a</sup> Epigenetic loci: loci where the contribution of one of the parental genomes (maternal or paternal) has a preferential or exclusive effect on the phenotype of the progeny

<sup>b</sup> Variance-components linkage analysis

<sup>c</sup> Genome-wide scan

<sup>d</sup> Analysed with obstetric cholestasis as cofactor

<sup>e</sup> Lodscore

loci (QTL). This led to the identification of the *ACVR2* gene and as confirmed in the Norwegian population (Moses et al. 2006; Tommerdal et al. 2006).

### Epigenetic inheritance in preeclampsia

A striking parallel exists in the epigenetic and other features between the 10q22 locus and the 2p13 locus associated with preeclampsia in Dutch and Icelandic females, respectively (Van Dijk et al. 2005; Arngrimsson et al. 1999; Oudejans et al. 2004). Within a large region in both chromosomes, shared features include the presence and similar line-up of paralogous genes (*EGR2-CTNNA3-HK1-TACR2*), genes predicted to be imprinted (*LOXL3, DOK1, HK2, TACR1*) and other features (*FRA10D, FRA2E*) (Table 3) (Luedi et al. 2005). The 2p13 locus previously found to segregate with two large Icelandic families has now been confirmed to be associated with the Finnish populations as well (Arngrimsson et al. 1999; Laasanen et al. 2003). Ongoing searches for the 2p13 gene are likely to be successful if these similarities are taken into account, i.e. by searching for placentally expressed genes on 2p13 potentially subject to epigenetics within the region of 10q22 and 2p13 with common evolutionary origin. The region on 2p13 flanked by D2S2111-D2S1777 fulfills these criteria (Table 3).

### Genetics of polyploidy

The variant *STOX1* gene carried and transmitted by Dutch preeclamptic females has been predicted to induce premature trophoblast differentiation (Van Dijk et al. 2005). By premature differentiation from diploid, invasive into polyploid, non-invasive giant cells, the process normally controlled by these cells, establishment of feto-maternal vascular connection, becomes defective by a partial failure in trophoblast

invasion (Van Dijk et al. 2005; Pijnenborg et al. 2006). Giant cell transformation is also a normal feature of liver cells and megakaryocytes, i.e. the cells predominantly affected in the HELLP syndrome. This suggests parallels in control. However, as explained above, the HELLP syndrome is placental in origin. This indicates that, if parallels in genetic control of polyploidy exist, they are indirect. Support for this exists. Molecules (e.g. soluble FLT1 receptors) that prevent the VEGF-FLT1 interaction on the membrane of precursor megakaryocytes significantly inhibit polyploidization, while addition of exogenous VEGF or PlGF markedly potentiates megakaryocyte maturation (Casella et al. 2003). These data confirm a potential relation between polyploidy, the cells affected in HELLP and the effector molecules of preeclampsia and warrant further exploration. Secondly, genetic linkage analysis has shown that the HELLP syndrome is a distinct genetic entity. In fact, discrimination of preeclampsia patients without HELLP and those with HELLP only was essential in the Dutch genome-wide linkage analysis (Lachmeijer et al. 2001). In Dutch females, the 12q24 region is linked with HELLP. As no epigenetic parallels exist between 12q24 and 10q22/2p13, and given the existence of discordant monozygous twin sisters, the HELLP susceptibility gene is likely to involve a placentally expressed gene inherited in an autosomal recessive manner by the child.

### The paraHOX paralogon

Under a strict diagnostic model, the 4q34 region between D4S450-D4S610 was identified in the Australian/New Zealand cohort by parametric linkage analysis (Harrison et al. 1997). Using the same strict diagnostic criteria, but following variance-components-based linkage, two additional susceptibility loci were identified on 5q (D5S644) and 13q (D13S173) (Johnson et al. 2006). Interestingly, these three regions, all associated with the Australian/New Zealand loci, coincide with the paraHOX paralogon cluster (Fredriksson et al. 2003). Phylogenetic analysis of the superfamily of G-protein coupled receptors identified four paralogon regions, 4q, 5q, 13q and X, the so-called paraHOX paralogon, enriched for G-protein receptors of the  $\beta$ -group of rhodopsin receptors (Fredriksson et al. 2003). This cluster contains the endothelin-related receptors *EDNRA* and *EDNRB*. In addition, the 4q region contains the *STOX2* gene. These parallels could assist in the identification of the susceptibility genes associated with preeclampsia in specific populations.

**Table 3** Features shared between the 10q22 and 2p13 loci

Locus	10q22	2p13
Syndrome	Preeclampsia	Preeclampsia
Population	Netherlands	Iceland
Parent-of-origin effect	Maternal	Likely
Recombination hotspot	Female	Yes
DNA/RNA cluster	Yes	Yes
Paralogs	Yes	Yes
Fragile sites	FRA10D	FRA2E
Selective sweep	Yes	Yes
Gene	STOX1	D2S2111-D2S1777

## Malice in wonderland

The majority, if not all, loci associated with preeclampsia, when grouped according to disease entity or epigenetic features, share phylogenetic features reflecting a common evolutionary origin and/or function. In other words, the conserved genetic functions underlying the development of hemochorial placentation in mammals in general, and in humans in particular, are mirrored in the genetics of preeclampsia.

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