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ARTICLE

Association between the candidate susceptibility gene *ACVR2A* on chromosome 2q22 and pre-eclampsia in a large Norwegian population-based study (the HUNT study)

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Genome-wide scans in Icelandic, Australian/New Zealand and Finnish pedigrees have provided evidence for maternal susceptibility loci for pre-eclampsia on chromosome 2, although at different positions (Iceland: 2p13 and 2q23, Australia/New Zealand: 2p11–12 and 2q22, Finland: 2p25). In this project, a large population-based ($n = 65\,000$) nested case–control study was performed in Norway to further explore the association between positional candidate genes on chromosome 2q and pre-eclampsia, using single-nucleotide polymorphisms (SNPs). DNA samples from 1139 cases (women with one or more pre-eclamptic pregnancies) and 2269 controls (women with normal pregnancies) were genotyped using the Applied Biosystems SNPlex high-throughput genotyping assay. In total, 71 SNPs within positional candidate genes at 2q22–23 locus on chromosome 2 were genotyped in each individual. Genotype data were statistically analysed with the sequential oligogenic linkage analysis routines (SOLAR) computer package. Nominal evidence of association was found for six SNPs (rs1014064, rs17742134, rs1424941, rs2161983, rs3768687 and rs3764955) within the activin receptor type 2 gene (*ACVR2A*) (all *P*-values < 0.05). The non-independence of statistical tests due to linkage disequilibrium between SNPs at a false discovery rate of 5% identifies our four best SNPs (rs1424941, rs1014064, rs2161983 and rs3768687) to remain statistically significant. The fact that populations with different ancestors (Iceland/Norway–Australia/New Zealand) demonstrate a common maternal pre-eclampsia susceptibility locus on chromosome 2q22–23, may suggest a general role of this locus, and possibly the *ACVR2A* gene, in pre-eclampsia pathogenesis.
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Introduction

Pre-eclampsia is a complex and potentially life-threatening disorder affecting approximately 3% of all pregnant women in the western world.[1] Pre-eclampsia typically develops in the latter half of pregnancy and is a rapidly progressive condition characterized by hypertension and

proteinuria. Although the condition has been known for a long time, pre-eclampsia is still one of the major pregnancy disorders and a leading cause of maternal and fetal morbidity and mortality.[2] There is no effective treatment, and delivery of the baby is the only alternative if the life of the pregnant woman is threatened.

Epidemiological research through decades has demonstrated that pre-eclampsia has a familial association, and this has led to the conclusion that genetic control and inheritance has a major function in the pathology of pre-eclampsia.[3] Genetic factors are suggested to be responsible for more than 50% of the liability to pre-eclampsia,[4,5] but the pattern of inheritance is still unclear. Although the tendency for pre-eclampsia to be passed on from mother to daughter was the first pattern to be observed,[3,6,7] it is now clear that fathers also contribute to pre-eclampsia susceptibility in their partners, possibly through paternal transmission of genes on to the fetus.[8,9]

The fact that genetic factors have a major function in the development of pre-eclampsia has encouraged several research groups to search for maternal pre-eclampsia susceptibility genes. New molecular technologies and better bioinformatics tools have become important approaches in the search for genetic clues in 'complex traits' such as pre-eclampsia. Genome-wide linkage scans of pre-eclampsia pedigrees have pointed to several maternal pre-eclampsia susceptibility loci.[10–14] Three independent and distinct studies from Iceland, Australia/New Zealand and Finland have shown linkage to chromosome 2 although at different positions.[10,13,14] By the use of a variance components-based linkage approach, the Australian research group resolved a susceptibility quantitative trait locus for pre-eclampsia on chromosome 2q22, and an objective prioritization strategy assigned the highest priority to the activin receptor type 2 gene (*ACVR2A*),[4] which is a receptor for the cell signalling protein activin A. Activin A is a multifunctional protein with roles in cell differentiation, implantation[15] and decidualization.[16] In addition, recent studies suggest that activin A is involved in pathogenesis of inflammatory diseases[17–19] and atherogenesis.[20,21]

In this study, the association between the positional candidate genes at the 2q22 susceptibility locus, among them *ACVR2A,* has been explored further in a large Norwegian case–control cohort, using association analysis of SNPs. In total, 1139 cases (women with one or more pre-eclamptic pregnancies) and 2269 controls (women with normal pregnancies) were genotyped.

Materials and methods

Study population

All women in this study were retrospectively recruited from Nord-Trøndelag County in Norway as part of a large multipurpose health survey conducted during 1995–1997 (the HUNT2 study). More than 65 000 inhabitants participated. The people living in Nord-Trøndelag County are considered representative for the Norwegian population, and is well suited for genetic studies because of its ethnic homogeneity (<3% non-Caucasians).[22,23] The study population is described in detail elsewhere.[24]

Pre-eclampsia diagnosis and identification of cases

Pre-eclampsia was defined as the onset of persistent hypertension (exceeding 140/90 mmHg), in combination with proteinuria (exceeding 300 mg/day) after the 20th week of gestation. The women with pre-eclamptic and normal pregnancies in the HUNT cohort were identified by linking the HUNT database to the database at the Medical Birth Registry of Norway (MBRN), using diagnosis codes ICD-8 (before 1998) and ICD-10 (after 1998).

We identified 1179 women registered with pre-eclampsia in one or more pregnancies (cases) and 2358 women with a history of normal pregnancies as controls. Among these, blood samples were available from 1139 women from the case group and 2269 women from the control group at the HUNT biobank. Finally, cases were subdivided into subgroups; 1003 women were registered with one pre-eclamptic pregnancy, 606 of these had pre-eclampsia in their first pregnancy, whereas 397 had pre-eclampsia in a later pregnancy. One hundred and thirty-six women had experienced pre-eclampsia in more than one pregnancy.

Chromosome 2q candidate genes and SNP prioritization

The identification and objective prioritization of positional candidate genes at the Australian/New Zealand chromosome 2q pre-eclampsia susceptibility locus has been described previously.[4] In this study, seven positional candidate genes at this locus were selected for SNP-specific association analysis on the basis of their GeneSniffer 'hit-scores' (www.genesniffer.org), differential gene expression levels, preliminary SNP association and linkage disequilibrium (LD) data and/or scientific literature pertaining to their potential function in reproductive biology.[4,25–27] The seven positional candidate genes selected are: chemokine (C-X-C motif) receptor 4 (*CXCR4)*, histamine *N*-methyltransferase (*HNMT*), kynureninase (L-kynurenine hydrolase) (*KYNU*), reprimo (*RPRM*), activin A receptor type I (*ACVR1),* activin A receptor type IC (*ACVR1C*) and activin A receptor type 2 (*ACVR2A*). Seventy-one validated and/or coding sequence SNPs within these genes were either selected from the NCBI SNP database (dbSNP) (human build 36.2), identified by re-sequencing of candidate genes in the Australian/New Zealand pre-eclampsia pedigrees (E Fitzpatrick, personal communication) or obtained from the published literature[28] (Table 1).

SNP genotyping

DNA for genotyping was extracted from blood samples stored in the HUNT biobank as described elsewhere.[24] The

Table 1 Objectively prioritized chromosome 2q positional candidate SNPs

Gene	*SNP*	*Location (bp)*	*Genetic location*	*Function*	*Allele frequency*		P-*value (MG)*	*OR (95% CI)*
CXCR4	rs2734871	136 586 343	Genomic		C 0.7957	T 0.2043	NS	
	rs2228014	136 589 554	Exonic		C 0.9746	T 0.0254	NS	
	rs1051557	136 589 623	Exonic	Synonymous	NP			
	rs2680880	136 590 019	Intronic		T 0.6125	A 0.3875	NS	
	R334X[a]	136 590 140	Exonic	Nonsense	NP			
	E343X[a]	136 590 167	Exonic	Nonsense	NP			
	rs3806501	136 593 539	Genomic		NP			
	rs4954391	136 600 293	Genomic		T 0.7932	C 0.2068	NS	
HNMT	rs1471003	138 441 472	Intronic		NP			
	rs1982494	138 445 583	Intronic		T 0.6269	C 0.3731	NS	
	rs3791244	138 448 235	Genomic		C 0.8625	T 0.1375	NS	
	rs3100725	138 459 636	Intronic		C 0.7951	T 0.2049	NS	
	rs3113222	138 472 267	Genomic		NP			
	rs1801105	138 476 119	Genomic		C 0.8668	T 0.1332	NS	
	rs3100719	138 480 404	Genomic		C 0.7685	T 0.2315	NS	
	rs4954941	138 486 437	Genomic		G 0.7902	A 0.2098	NS	
KYNU	rs16858172	143 352 974	Intronic		C 0.9597	A 0.0403	NS	
	rs1439875	143 355 039	Intronic		T 0.9515	G 0.0485	NS	
	rs16858196	143 356 117	Intronic		NP			
	rs4662304	143 358 120	Genomic		G 0.5119	A 0.4881	NS	
	rs16858205	143 359 529	Exonic	Synonymous	NP			
	rs1438266	143 363 789	Intronic		A 0.5138	G 0.4862	NS	
	rs6429992	143 379 037	Intronic		T 0.5112	C 0.4888	NS	
	rs351675	143 428 514	Intronic		G 0.9927	C 0.0073	NS	
	rs2304705	143 431 735	Exonic	Missense	NP			
	rs1050951	143 463 434	Exonic	3′UTR	G 0.8966	A 0.1034	NS	
	rs2304700	143 507 484	Genomic		G 0.8907	T 0.1093	NS	
	rs9013	143 514 659	Genomic		T 0.8435	C 0.1565	NS	
ACVR2A	rs1424954	148 317 264	5′UTR		G 0.7247	A 0.2753	0.387	0.94 (0.82–1.07)
	rs13224	148 319 154	5′UTR		NP			
	rs7572676	148 320 092	Intronic		NP			
	LF002[b]	148 328 072	Intronic		NP			
	rs1014064	148 328 624	Intronic		A 0.7176	G 0.2824	0.0184	0.86 (0.77–0.98)
	rs17741978[b]	148 333 850	Intronic		C 0.8267	G 0.1733	0.0689	1.14 (0.99–1.32)
	rs1895694	148 336 216	Intronic		A 0.5240	G 0.4760	0.918	1.00 (0.90–1.11)
	rs2113794	148 339 447	Intronic		A 0.5235	C 0.4765	0.998	1.01 (0.90–1.12)
	rs17742134[b]	148 343 866	Intronic		C 0.8252	T 0.1748	0.0214	1.17 (1.02–1.35)
	rs1424941	148 359 588	Intronic		G 0.8235	A 0.1765	0.0171	1.18 (1.03–1.36)
	rs2161983	148 365 856	Intronic		C 0.7139	T 0.2861	0.0196	0.86 (0.77–0.98)
	rs2288190	148 374 059	Intronic		C 0.6428	T 0.3572	0.0554	0.89 (0.79–0.99)
	rs10497025[b]	148 378 672	Intronic		C 0.7618	G 0.2382	0.0586	0.88 (0.78–1.00)
	rs1227307	148 378 775	Intronic		NP			
	rs3768687	148 388 490	Intronic		G 0.7162	A 0.2838	0.0214	0.86 (0.76–0.98)
	LF020[b]	148 390 323	Intronic		A 0.9946	G 0.0054	0.787	0.81 (0.29–2.24)
	rs3764955	148 391 267	Intronic		C 0.7208	G 0.2792	0.0327	0.87 (0.77–0.98)
	rs7601098	148 393 084	Intronic		G 0.9380	C 0.0620	0.538	1.08 (0.86–1.34)
	rs2303392	148 396 897	Intronic		Failed			
RPRM	rs3770303	154 041 809	3′ near gene		C 0.8265	T 0.1735	NS	
	rs1052581	154 042 352	3′UTR		G 0.9141	A 0.0859	NS	
	rs1063728	154 042 745	3′UTR		G 0.5289	C 0.4711	NS	
	rs2033764	154 044 214	Genomic		A 0.8134	G 0.1866	NS	
ACVR1C	rs6751595	158 097 764	3′UTR		NP			
	rs7594480	158 098 714	Exonic	Missense	T 0.9349	C 0.0651	NS	
	rs6746788	158 109 351	Exonic	Synonymous	NP			
	rs4556933	158 152 135	Exonic	Synonymous	G 0.6425	A 0.3575	NS	
ACVR1	rs3738927	158 302 306	Exonic	Synonymous	NP			
	rs6729964	158 330 839	Exonic	Synonymous	NP			
	rs1146031	158 335 226	Exonic	Synonymous	A 0.9949	G 0.0051	NS	
	rs2227861	158 345 156	Exonic	Synonymous	A 0.7437	G 0.2563	NS	
	rs13406336	158 364 208	Exonic	Missense	G 0.9970	C 0.0030	NS	
	rs16842103	158 367 073	Intronic		NP			
	rs16842105	158 367 916	Intronic		NP			
	rs7561419	158 370 035	Intronic		NP			
	rs17798043	158 371 523	Intronic		C 0.9130	T 0.0870	NS	
	rs16842106	158 371 886	Intronic		NP			
	rs1036739	158 373 442	Intronic		C 0.7790	T 0.2210	NS	
	rs10497191	158 375 463	Intronic		C 0.8717	T 0.1283	NS	
	rs4380178	158 376 691	Intronic		Failed			
	rs4294956	158 376 700	Intronic		Failed			
	rs12327948	158 377 663	Intronic		C 0.8047	G 0.1953	NS	
	rs1965812	158 379 544	Intronic		C 0.8071	T 0.1929	NS	
	rs10497192	158 379 946	Intronic		T 0.7024	C 0.2976	NS	

NP; non-polymorphic, *P*-value (MG); *P*-value for the measured genotype test, OR; odds ratio, CI; confidence interval, NS; not significant, SNPs; single-nucleotide polymorphisms.
[a]Hernandez *et al*.[28]
[b]SNP discovery in Australian/New Zealand pre-eclampsia cohort (E Fitzpatrick, personal communication).

Applied Biosystems SNPlex™ Genotyping System (Applied Biosystems, CA, USA) was used for SNP genotyping. All samples were electrophoretically separated on a 3730 DNA Genetic Analyzer (Applied Biosystems), and automated allele calls and genotype clustering of each individual sample was performed by Applied Biosystems' GeneMapper® Software (version 4.0), as described in more detail elsewhere.[24]

Statistical analysis

Positional candidate SNPs were analysed in SOLAR[29] using a measured genotype test.[30] The analysis used a standard threshold model assuming an underlying normal distribution of liability. The threshold model and its assumptions are virtually identical to those used in standard logistic regression but benefits from the ease of interpretation with regard to genetic effects. The measured genotype test of associations assesses the extent of genotypic mean differences (on the liability or risk scale) between case and control singletons assuming a model of additive gene action. Odds ratios (ORs) with 95% confidence intervals were calculated using SPSS 13.0 (for Mac OS® X) under the assumption of an additive genetic model. LD parameters among intragenic SNPs were estimated using basic correlation methods to assess all disequilibria jointly using SOLAR. To accommodate for multiple hypothesis testing, we used the approach of Li and Ji[31] to determine the effective number of independent SNPs (and tests) in combination with applying a false discovery rate (FDR) of 5%.[32]

Ethics

The study was approved by the Regional Committee for Medical Research Ethics, Norway and approved by the National Data Inspectorate and The Directorate of Health and Social Welfare. Ethical approval for genotyping and statistical analysis of the Norwegian cohort was obtained from The University of Texas Health Science Center at San Antonio, Institutional Review Board.

Results

Statistical power analysis

Formal power calculations using SOLAR determined that for the case and control sample available for study, there was an 80% likelihood of identifying a SNP accounting for at least 2% of the total variation in the dichotomous pre-eclampsia phenotype (where affected is scored as 1 and unaffected as 0).

SNP genotyping and association

Twenty of the 71 gene-centric SNPs (15 coding, 56 non-coding) were non-polymorphic and three additional SNPs failed the SNPlex genotyping assay (Table 1). All successfully genotyped polymorphic SNPs conformed to Hardy–Weinberg proportions ($P>0.05$).

Of the seven genes tested, we observed nominal SNP associations pertaining to *ACVR2A* only (Table 1). There were a total of 19 SNPs tested in *ACVR2A* with six SNPs (rs1014064, rs17742134, rs1424941, rs2161983, rs3768687 and rs3764955) exhibiting nominal association with the pre-eclampsia group ($n=1139$) ($P<0.05$). An additional three SNPs (rs17741978, rs2288190 and rs10497025) exhibited borderline significance ($P<0.07$), whereas another five SNPs were nonsignificant (Table 1). The remainder of the tested *ACVR2A* SNPs were either non-polymorphic ($n=4$) or failed the SNPlex genotyping assay ($n=1$). Analysis of the *ACVR2A* genotypes of subgroups of cases (women registered with one pre-eclamptic pregnancy ($n=1003$), women with pre-eclampsia in their first and only registered pre-eclamptic pregnancy ($n=606$), women with pre-eclampsia once but in a later pregnancy ($n=397$) and women experienced pre-eclampsia in more than one pregnancy ($n=136$)) were compared to controls but there were no significant differences between the subgroups (data not shown). Thus, there is no evidence of locus-specific genetic heterogeneity associated with variations in the definition of the disease.

Of the SNPs displaying a nominal or borderline association with pre-eclampsia, we observed discordant OR either greater than one (rs17741978, rs17742134 and rs1424941) or less than one (rs1014064, rs2161983, rs2288190, rs10497025, rs3768687 and rs3764955) (Table 1). In our calculations an $OR>1$ is indicative of pre-eclamptic women more likely than control women to carry the minor allele at a tested *ACVR2A* SNP locus, whereas an $OR<1$ is indicative of pre-eclamptic women less likely than control women to carry the minor allele, hence favouring the major allele, at a tested *ACVR2A* SNP locus.

All polymorphic *ACVR2A* SNPs were re-tested in SOLAR with the inclusion of maternal age as a covariate effect. Nominal and borderline associated *ACVR2A* SNPs did not differ when adjusted for maternal age (data not shown). OR for nominal and borderline *ACVR2A* SNPs associated with pre-eclampsia did not differ either when adjusted for maternal age (data not shown).

The re-evaluation of our statistical results for *ACVR2A* to take into account multiple testing and the non-independence of the tests due to LD between SNPs utilized 13 of the 14 tested SNPs. The LF020 SNP was eliminated because of the observed low number of copies preventing valid use of our statistical test. Of the 13 remaining SNPs, we identified the effective number of independent SNPs and therefore statistical tests to be five using the approach by Li and Ji.[31] Five tests is a substantial reduction from the original 13 and when a modified FDR approach is incorporated we find that the four best SNPs (rs1424941, rs1014064, rs2161983 and rs3768687) meet an FDR of 0.05. Therefore, allowing for the total experiment, these four SNPs are

significant and we have only a 1 in 20 chance of having made a false discovery. FDR approaches can be substantially more powerful than classical approaches (eg, Bonferroni) for multiple testing.[32]

None of the tested SNPs within the other six genes (*CXCR4*, *HNMT*, *KYNU*, *RPRM*, *ACVR1* or *ACVR1C*) exhibited an association with pre-eclampsia (Table 1).

ACVR2A SNP LD

Figure 1 portrays the overall pattern of LD exhibited among *ACVR2A* SNPs. The patterns of LD are measured by the squared value of the pairwise correlation among intragenic genotypes (r^2). Hence, observed patterns of LD are herein referred to as isocorrelated redundant variant (IRV) sets.[33] Among the typed *ACVR2A* SNPs, we observe three very strong ($r^2 > 0.7$) IRV sets, namely, IRV set 1 (IRV1); rs1424954, rs1014064, rs2161983, rs2288190, rs10497025, rs3768687 and rs3764955, IRV2; rs17741978, rs17742134 and rs1424941, IRV3; rs1895694 and rs2113794. The SNP loci within IRV1 all exhibit an OR < 1, hence pre-eclamptic women are more likely than control women to carry the major allele at these *ACVR2A* loci. The SNP loci within IRV2 all exhibit an OR > 1, hence pre-eclamptic women are more likely than control women to carry the minor allele at these *ACVR2A* loci.

Discussion

In the present large positional candidate gene-based association study, the existence of a maternal susceptibility locus for pre-eclampsia at chromosome 2 was verified in Norwegian women with nominal association being obtained for multiple SNPs within the activin receptor gene *ACVR2A* on 2q22. When we accounted for multiple hypothesis testing and the non-independence of genotyped SNPs, our four best *ACVR2A* SNPs (rs1424941, rs1014064, rs2161983 and rs3768687) remained significantly associated with pre-eclampsia. For some of these SNPs it was the minor allele that was associated with pre-eclampsia, whereas for other SNPs it was the major allele, most likely reflecting naturally occurring evolutionary processes such as frequency differences, history and origins of each *ACVR2A* SNP locus.

Although replication of an association with the *ACVR2A* gene across non-related populations supports the hypothesis

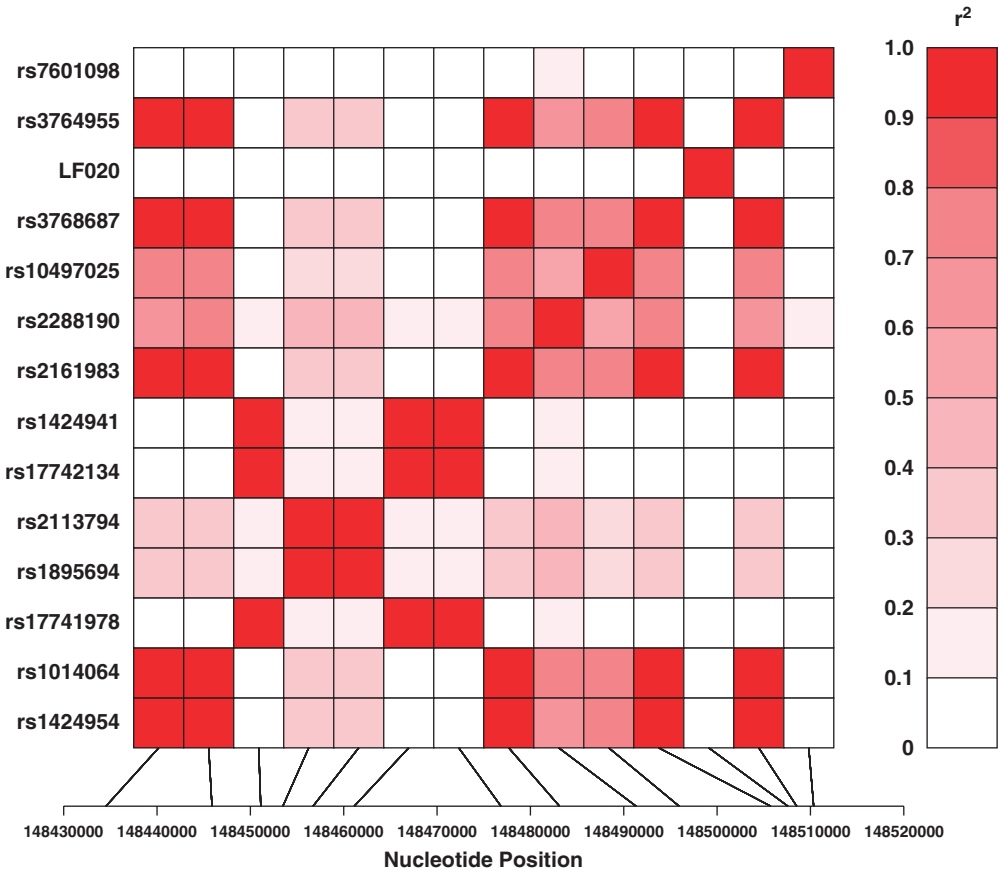

Figure 1 The pattern of linkage disequilibrium in *ACVR2A*. The intensity of red colour depicts the magnitude of the squared value of the pairwise correlation among intragenic genotypes. The more intense colour ($0.0 \geq r^2 \geq 1.0$) the stronger the correlation.

that genes influencing maternal susceptibility for development of pre-eclampsia are located at chromosome 2q22, we could not see concordance between the tested *ACVR2A* SNPs showing association in our Norwegian and Australian/New Zealand pre-eclampsia cohorts. However, we do observe strong LD between the SNP showing the strongest association in the Australian study (rs1424954) and four SNPs (rs1014064, rs2161983, rs3768687, rs3764955) showing nominal association with pre-eclampsia in the Norwegian cohort. Unfortunately, this SNP was relatively poorly typed in the Norwegian sample and the reduced sample size ($n = 2274$) is a likely reason for the weaker association result. Nonetheless, these data continue to bring focus on a possible role for the *ACVR2A* gene in pre-eclampsia pathogenesis.

Activin belongs to the transforming growth factor-β (TGF-β) family that can regulate cell differentiation, proliferation and apoptosis,[34,35] and utilizes two types of cell surface receptors (ACVR1 and ACVR2). The receptors consist of an extracellular domain that specifically binds activin A, a membrane-spanning domain and an intracellular kinase domain.[36] The *ACVR2A* gene encodes the activin A type 2 receptor.[36] Potential ligands for ACVR2 include activin A, activin B and inhibin A, but not TGF-β.[37] Activin A is secreted by most cell types, and control of expression of both receptors appears to occur in a tissue- and gene-specific manner during human development.[38] Activin receptors are expressed in human placental tissue (trophoblasts and vascular endothelial cells) from early pregnancy.[39] Activin A promotes trophoblast proliferation and differentiation[15] and appears to have a fundamental function during implantation and decidualization.[40] In addition, placental hormonogenesis[41] and uterotonin secretion[42] are modulated by activin A.

The elevated levels of activin A have been found in pre-eclamptic women.[43–48] Placenta is probably the main source of activin A in maternal circulation.[49] Altered expression of *ACVR2A*, as suggested by the gene expression analysis performed by Moses *et al*,[4] may influence activin A concentrations and action with consequences on trophoblast invasion and remodelling of the spiral arteries. However, it is not clear whether the increased activin A concentration/reduced *ACVR2A* expression is a cause or a consequence of placental dysfunction. Thus, the activin pathway is a plausible biological candidate for a role in mechanisms perpetuating pre-eclampsia pathogenesis. Recently, activin A has been suggested to have a significant function in inflammatory diseases[17–19] and atherogenesis.[20,21] The elevated serum levels of activin A have been reported in patients with cardiovascular diseases (CVD), and a downregulated expression of *ACVR2A* has been shown in patients with unstable CVD, suggesting a dysregulated receptor expression.[50] Women with pre-eclampsia have an increased morbidity and mortality of CVD.[51] Thus, the hypothesis may be raised that the linkage between *ACVR2A* and pre-eclampsia may be ascribed to the role of activin A in endothelial activation and systemic endovascular inflammatory responses. Supporting the inflammatory response hypothesis, we recently reported a significant association between the *SEPS1* gene (which is involved in stress response in the endoplasmic reticulum and inflammation control) and pre-eclampsia in our Norwegian cohort.[24]

It is also plausible that our association signals are not the result of a causal variation involving the *ACVR2A* gene but are acting as markers for causal variation in a nearby gene(s). To investigate this possibility we assessed other genes within 500 kb of *ACVR2A*. The origin recognition complex, subunit 4-like (yeast) (*ORC4L*) gene is the only gene within this chromosomal region and resides telomeric to *ACVR2A* with ~300 kb separating their respective 3′UTRs. Utilizing the Australian/New Zealand data set, there is evidence of strong LD ($0.74 \geq r^2 \geq 1.00$) between typed *ACVR2A* and *ORC4L* SNPs (unpublished data). The protein encoded by *ORC4L* is a subunit of the ORC complex, which is essential for DNA replication in mammalian cells.[52] Without complete deep re-sequencing of *ACVR2A* and *ORC4L* in the Norwegian sample, followed by functional assessment of the prioritized variants showing association (which is beyond the scope of this current study), we can only speculate as to the potential role of the genetic signals observed in this genomic region, either by *ACVR2A* and/or *ORC4L*, in reference to pre-eclampsia susceptibility.

In conclusion, we have shown evidence for association between the *ACVR2A* gene and pre-eclampsia in a Norwegian population. By this, we have confirmed data obtained in the Australian/New Zealand family linkage study.[4] The determination of the actual functional polymorphisms in *ACVR2A* and nearby genes that confer susceptibility to pre-eclampsia remains the challenge ahead. The Australian group performed whole genome microarray analysis of decidual tissues collected from pre-eclamptic and normotensive pregnant women. They observed 15 of the 17 transcripts at 2q with at least a 2.5-fold change in expression being downregulated in the pre-eclamptic samples, compared to normotensive tissue.[4] Among these 15 transcripts, the *ACVR* genes (both *ACVR2* and *ACVR1C*) demonstrated a more than 10-fold difference. However, expression analysis was performed on a rather small collection of samples (seven cases and seven controls), and the individual samples of each group were pooled before expression analysis was performed.[4] We propose to perform further transcriptional profiling on a large Norwegian collection of decidual samples from pre-eclampsia cases and controls, with associated genotyping of the *ACVR2A* gene in individuals that show differential decidual expression of the gene. By using this approach, we hope to gain information about a possible causal link between *ACVR2A* genotype(s) and the

expression of this gene. In addition, transcription-based approaches may imply a more appropriate prioritization of candidate genes in future epidemiological studies of genetics in pre-eclampsia.

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