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Evidence for a Familial Pregnancy-Induced Hypertension Locus in the eNOS-Gene Region

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Summary

Pregnancy-induced hypertension may be regarded as a manifestation of endothelial-cell dysfunction. The role of the eNOS gene in the development of a familial pregnancy-induced hypertension was evaluated by analysis of linkage among affected sisters and in multiplex families ($n = 50$). Markers from a 4-cM region encoding the eNOS gene showed distortion from the expected allele sharing among affected sisters ($P = .001–.05$), and the statistic obtained from the multilocus application of the affected-pedigree-member method also showed distortion ($T_{[f(P)=\mathrm{sqrt}(P)]} = 3.53$; $P < .001$). A LOD score of 3.36 was obtained for D7S505 when a best-fitting model derived from genetic epidemiological data was used, and LOD scores of 2.54–4.03 were obtained when various other genetic models were used. Estimates of recombination rate, rather than maximum LOD-score values, were affected by changes in the genetic parameters. The transmission-disequilibrium test, a model-free estimate of linkage, showed strongest association and linkage with a microsatellite within intron 13 of the eNOS gene ($P = .005$). These results support the localization of a familial pregnancy-induced hypertension–susceptibility locus in the region of chromosome 7q36 encoding the eNOS gene.

Introduction

Pregnancy-induced hypertension or preeclampsia syndrome is a major cause of maternal and fetal morbidity and mortality. The majority of cases are sporadic, but a familial pregnancy-induced hypertensive disorder has been described (Cooper and Liston 1979; Chesley and Cooper 1986; Arngrímsson et al. 1990). Clinical manifestation of the condition can range from mild disease with transient hypertension in the latter part of pregnancy to life-threatening illness with convulsions in hypertensive pregnancy, thrombocytopenia, HELLP (hemolysis, elevated liver enzymes, and low platelets) syndrome, and fetal hypoxia and growth retardation. Most of the pathophysiological changes, such as hypertension, volume contraction, platelet exhaustion, disseminated intravascular coagulation, and enhanced vascular reactivity, can be related to endothelial-cell dysfunction (Roberts and Redman 1993). The endothelial dysfunction could be mediated by circulating factor(s) originating from the placenta. Inherent abnormalities in the vascular endothelium are equally likely to be a contributing cause in the familial form of the disease. In both situations abnormalities in a final common pathway, leading to impairment of endothelial function, would be anticipated.

Constitutive nitric oxide (NO) production in endothelial cells increases during pregnancy and contributes to vasodilatation and blunting of vasopressor response (Goetz et al. 1994; Nathan et al. 1995). Evidence for the role of NO in the pathogenesis of pregnancy-induced hypertension comes from both animal and human studies. In women developing pregnancy-induced hypertension NO generation has been shown to be inappropriately low (Delacretaz et al. 1995), and administration of a NO donor improves flow in the uterine artery in normal early pregnancy and in women at high risk of developing the disease (Ramsay et al. 1994). Inhibition of NO synthesis in animals during pregnancy produces hypertension, proteinuria, thrombocytopenia, and fetal growth retardation (Yallampalli and Garfield 1993; Molnar et al. 1994). These findings make the endothelial NO synthase (eNOS) gene a primary candidate for the familial pregnancy-induced hypertension syndrome.

Subjects, Material, and Methods

Families and Status Definition

The concept of familial pregnancy-induced hypertension was first described by Kaku and Nagata (1955) and Browne and Scheumack (1956), who suggested that the

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family form might unmask a tendency toward hypertension later in life. Later, Humphries (1960), Cooper and Liston (1979), and Chesley and Cooper (1986) further defined the condition. Although in the original descriptions of the families the probands invariably had severe disease defined as both elevated blood pressure (>140/90 mmHg) in pregnancy and proteinuria (then most often called "severe preeclampsia"), a considerable proportion of other female members of the families who were classified as affected had only hypertension without proteinuria. For example, in the study by Chesley and Cooper (1986) nearly half of the affected women did not have any recording of proteinuria and were classified as having mild preeclamptic disease. It has been suggested that having only one component of the syndrome is not diagnostic, but its absence never excludes the diagnosis with certainty (Redman 1989). Therefore it can only be claimed that women with a single sign of the syndrome, such as gestational hypertension alone, may possibly have the syndrome.

"Preeclampsia," "preeclamptic toxemia," "pregnancy-induced hypertension," "hypertensive disease of pregnancy," and "gestosis" are roughly synonymous terms describing various aspects of transient hypertension in late gestation. The condition cannot at the moment be stereotyped, and the balance of its different components will vary from case to case. One woman may have severe hypertension but little renal involvement, another severe renal involvement but little hypertension, and a third predominantly hepatic involvement. It may even be possible to have preeclampsia without hypertension (Redman et al. 1977). The simplest definition of the syndrome is therefore a pregnancy-specific syndrome characterized by a group of signs of which at least two must be present (Redman 1989). These could, for example, be either hypertension and proteinuria or hypertension and convulsions, since eclampsia can occur without proteinuria (Sibai et al. 1981). Our classification scheme has taken the aforementioned considerations into account, and women were classified as having severe pregnancy-induced hypertensive disease if they developed during pregnancy at least one of the following clinical signs: proteinuria (equivalent to ≥1+ on dipstick), convulsions (eclampsia), or intrauterine growth retardation, in addition to gestational hypertension (diastolic blood pressure ≥90 mmHg after 20 wk of pregnancy). Hospital records were verified for all family members who fell into this category. First- or second-degree relatives of the probands who developed only a single symptom, such as hypertension, after 20 wk of pregnancy, as well as those who stated that they had developed the severe disease but for whom the relevant hospital records were not found, were classified as having mild or possibly pregnancy-induced hypertension. In this study, genotyping was performed on DNA samples from 106 women who fulfilled the above criteria for severe pregnancy-induced hypertensive disease and from 42 women with either mild or possibly pregnancy-induced hypertension who were from 50 families from Scotland and Iceland. Males were classified as unknown in all analyses. Women with blood pressure <90 mmHg during pregnancy were classified as normal. Women who fell into the mild-disease category were classified as affected in the first run of the statistical analysis, along with those with the severe condition, but were classified as unknown in the second analysis of the data. Outcome of first pregnancy was used to determine disease status. The diagnosis was based on information available at the time of the pregnancy, rather than on later follow-up health assessments with respect to either development of recurrent disease or tendency toward subsequent cardiovascular problems. No attempt was made to obtain confirmation of prepregnancy blood-pressure readings for these women (many had never had blood-pressure measurements done before their first pregnancy), and, as far as was possible, those with preexisting renal and hypertensive disease were excluded. Approval for the study was obtained from local ethics committees and the Icelandic Data Protection Committee.

Markers

Molecular-genetic analyses were performed by means of microsatellite amplifications using methods described elsewhere (Nadaud et al. 1994). A CA repeat from intron 13 of the eNOS gene was used in the first phase of the study, and in the second phase two flanking Généthon markers (D7S505/AFM199Zd4 and D7S483/AFM074Xg5) were added (Weissenbach et al. 1992). To ensure consistency, in the analysis of the PCR products, between laboratories, CEPH individual 1347.02 was genotyped, and these alleles were used for reference. This individual is heterozygous for the presence of 31 and 32 CA repeats in the eNOS gene, which are denoted alleles 13 and 14. At D7S505 he has alleles 1 and 5 (262/279 bp), at D7S483 alleles 8 and 9 (180/182 bp).

Mapping Strategy and Statistical Evaluation

The main effort of the collaborative group lies in linkage analysis of putative candidate genes for the familial pregnancy-induced hypertension syndrome. The strategy is based on analysis of highly informative microsatellites within or in the vicinity of the putative gene, followed by analyses of flanking markers if the initial analysis is suggestive of possible linkage. Because of the complexity of the genetics of pregnancy-induced hypertension, our main emphasis is on the use of nonparametric analytical tools (for which no assumption has to be made about the inheritance of the trait) to estimate evidence for linkage, followed by more-traditional, LOD-score calculations. The nonparametric arsenal for linkage analyses included sib-pair analysis identical by descent (IBD) as implemented in the SIBPAIR program

(Kuokkanen et al. 1996; Satsangi et al. 1996), the transmission-disequilibrium test (TDT) (Spielman et al. 1993; Terwilliger 1995), and the affected-pedigree-member (APM) method (Weeks and Lange 1992). In the SIBPAIR program the number of sib pairs is weighted according to the number of sibships (Blackwelder and Elston 1985). These methods were developed to detect distortion of independent segregation of the marker and a trait among affected relatives, without having to make assumptions about the mode of inheritance involved in the trait. When applied to sib pairs the sharing of 0, 1, or 2 alleles is estimated, as are sharing and nonsharing of parental alleles. In the latter application the expected 50% chance of sharing the parental allele IBD is compared with the observed values, by χ^2 calculations. More information is obtained from the data by use of this latter procedure than by the 0, 1, 2 method, because, if a parent is homozygous, the sib pair cannot be classified IBD by the 0, 1, 2 method, and the data are left out whereas the sharing/nonsharing data from the other parent (if heterozygous) can be fully informative. The APM method is based on the same principles, but it can be applied to extended or multiplex families. In this analysis identity by state (IBS), rather than the IBD method of comparison, is used, which makes it sensitive to allele-frequency estimation. It has therefore become a custom to use a different allele-weighting function in the analysis. In this study the results are presented with the f(p) = sqrt(p), function, as recommended, as well as with no weighting, f(p) = 1 (Weeks and Lange 1992).

The results were also analyzed with the TDT (Spielman et al. 1993). This test estimates the transmission of a given allele from heterozygous parents to their affected offspring. The TDT statistic is formulated as $(x - y)^2/(x + y)$, which is asymptotically distributed as a χ^2 statistic with 1 df. To perform the TDT analysis the TDTLIKE program was used (Terwilliger 1995). In the program, exact P values from binomial distribution were computed by a look at the probability of having the allele under study transmitted $\geq x$ times, of a total of $(x + y)$ opportunities, to the affected children of heterozygous parents. Also, correction for multiple testing has been implemented in the program. Therefore the P values presented reflect the probability that such results occur for one of the n alleles at a given locus, rather than assuming that only one single allele had been tested. Only alleles that have at least five opportunities to be transmitted were included, to reduce, in an unbiased way, the number of necessary tests to correct for. A likelihood ratio–based TDT test that considers all the alleles jointly, with one being transmitted preferentially to affected offspring and the other not being so transmitted, as implemented in the TDTLIKE program, was also used for linkage analysis (Terwilliger 1995). The probability that any given allele is transmitted is called "lambda" (λ) and is estimated by the maximum-likelihood method, where λ is constrained to be $>.5$ and $\lambda = .5$ is the null hypothesis. $-2\ln[L(\lambda)/L(\lambda = .5)]$ is assumed to be asymptotically distributed as a χ^2 statistic with 1 df. In multiplex families the TDT test can be used to test the hypothesis of no linkage, but only limited information about allelic association can be obtained.

LOD-score calculations using genetic parameters from previous genetic epidemiological studies were performed by use of the FASTLINK 2.3P version (Cottingham et al. 1993) of the LINKAGE package (Lathrop et al. 1984), first by use of parameters from our recent study, which supported multifactorial inheritance or a major autosomal dominant gene with low penetrance (i.e., the AD/LP model) (Arngrímsson et al. 1995). Second, other genetic models, derived from older data, were also used, since these have been applied in previous genetic-linkage studies: these models were as follows (q = gene frequency; f = penetrance): (1) autosomal recessive inheritance with complete penetrance—$q = .2$; $f_{A1A2} = f_{A2A2} = 1.0$ (Cooper and Liston 1979; Chesley and Cooper 1986); (2) autosomal recessive with incomplete penetrance—$q = .39$; $f_{A1A2} = f_{A2A2} = .41$) (Liston and Kilpatrick 1991); (3) a genetic model with partial dominance—$q = .1$; $f_{A1A2} = .21$ and $f_{A2A2} = 1.0$, derived from Arngrímsson et al. (1995); (4) an arbitrary autosomal dominant model with 90% penetrance—$q = .02$; $f_{A1A2} = f_{A2A2} = .90$; and (5) affecteds-only analysis—that is, a low-penetrance model for autosomal dominant inheritance—$q = .02$; $f_{A1A2} = f_{A2A2} = .01$. Two-point LOD-score calculations were performed by use of both equal frequency for all alleles at the marker locus and allele frequencies estimated from the families by use of the ILINK module of the linkage programs.

Results

Analysis of the eNOS Gene

A highly polymorphic microsatellite marker from intron 13 of the eNOS gene was used for genotyping (Nadaud et al. 1994). ASP analyses were performed by use of either only those affected women with the more secure diagnosis of severe pregnancy-induced hypertensive disease or all affected women (i.e., including also those with possible or mild disease). For both classification schemes, distortion from the expected 50:50 transmission of parental alleles was observed ($P < .001$–$.02$) (table 1). Analysis of sib pairs with one affected and one unaffected individual did not show any evidence of segregation distortion, with 27/28 sharing/nonsharing ($P = .48$). LOD-score calculations with equal allele frequency and using the AD/LP model ($q = .1$; $f = .3$) with sex-average recombination showed a maximum LOD score (Z_{max}) of 2.45 at theta (θ) = .08. This genetic model has previously shown the best goodness of fit to combined genetic epidemiological data from the United States, Scotland, and Iceland (Arngrímsson et al. 1995).

Table 1

Affected-Sib-Pair Analyses Using Chromosome 7 Markers

Marker	No. of Sib-Pairs Sharing[a]			No. of Sib Pairs in Whom Parental Alleles Are[b]		Significance	
	No Alleles	One Allele	Two Alleles	Shared	Not Shared	χ^{2} [c]	P
Severe or mild (possible) disorder:							
D7S505	4	11	12	52	30	5.90	.009
eNOS	9	24	19	67	44	4.77	.015
D7S483	7	12	15	52	34	3.77	.026
Severe disorder only:							
D7S505	1	7	9	37	15	9.31	.001
eNOS	5	15	12	44	26	4.63	.020
D7S483	5	8	10	34	21	3.08	.050

[a] Expected ratios are .25, .50, and .25 for no alleles, one allele, and two alleles, respectively.
[b] Expected shared:nonshared ratio is 50:50.
[c] Measurement of deviation from expected 50:50 shared:nonshared ratio.

When only information from the affected individuals (q = .1; f = .01) was used, Z_{max} = 2.63 at θ = .06 was obtained. When the recombination rate was allowed to vary with gender, Z_{max} increased to 2.73 at θ_f = .02, with a gender ratio of .1.

Analyses of eNOS and Flanking Markers

In light of the suggestive results from the eNOS(CA)$_n$ analysis, the two nearest flanking markers, D7S505 and D7S483, which map the eNOS gene to a 4-cM region on chromosome 7q36, were used for further analysis. Affected-sib-pair analyses of both these markers showed distortion from the expected 50:50 transmission of the parental alleles IBD, for both diagnostic-threshold levels used in the study. The significance level ranged from $P < .001$ to $P < .05$ (table 1). Two-point LOD-score analyses were performed for all three markers by use of allele frequencies estimated from the families and sex-averaged recombination. When the AD/LP model was used in the calculation, Z_{max} was found for marker D7S505—Z_{max} = 3.36 at θ = .00—followed by eNOS-(CA)$_n$, with Z_{max} = 2.44 at θ = .06 (table 2), which is similar to that estimated when equal frequency is assigned to each allele. The Z_{max} for D7S483 was 1.05 at θ = .14. When the analyses were restricted to women diagnosed in accordance with the more strict classification of severe pregnancy-induced hypertensive disease, with exclusion of women with a possible or mild form of the disease, the Z_{max} of 3.15 was observed for D7S505; for eNOS(CA)$_n$ Z_{max} = 1.95, and for D7S483 Z_{max} = 0.95 (table 2).

Effect of Genetic Parameters on LOD-Score Estimation

During the past 30 years several hypotheses have been put forward to explain the familial clustering of pregnancy-induced hypertension. The genetic models suggested have mainly involved some variation of autosomal recessive inheritance. We have therefore repeated the LOD-score calculations, using several genetic-parameter models for D7S505, which showed the strongest association in previous steps of the analysis. For these five models, Z_{max} = 2.54-4.03 at θ = .00–.16 (table 3). The Z_{max} of 4.03 was obtained by use of a model with partial dominance (q = .10; f_{A1A2} = .21 and f_{A2A2} = 1.0), which previously had shown slightly worse fit to the combined genetic epidemiological data from Iceland and Scotland than was seen with the AD/LP model (Arngrímsson et al. 1995). The lowest LOD score was found by use of an autosomal recessive model with reduced penetrance (q = .39; f = .41), which is an approximation for analysis of the maternal component of the Liston and Kilpatrick (1991) model, where it was suggested that the mother and fetus are homozygous for the same molecular aberration. Autosomal recessive inheritance with full penetrance (q = .20; f = 1.0) was suggested by Cooper and Liston (1979), and several publications have supported their findings (Chesley and Cooper 1986; Arngrímsson et al. 1990). Although, in the combined genetic epidemiological analysis, this model showed significantly worse fit than was seen with the AD/LP model, we have included this in our LOD-score calculations, because this model has been used in previous linkage studies (Wilton et al. 1990; Hayward et al. 1992; Arngrímsson et al. 1994). Z_{max} = 2.77 at θ = .16 was observed for this model, supporting possible localization of a familial pregnancy-induced hypertension susceptibility locus in the eNOS-gene region. LOD-score values were also calculated by use of an arbitrary autosomal dominant–gene model with high penetrance (q = .02; f = .9), under the assumption that we might be studying a relatively rare form of pregnancy-induced hypertension running in families. For this model, Z_{max}

Table 2

Analyses Using Genetic Parameters from Previous Genetic-Epidemiological Study (i.e., AD/LP Model [q = .10; f = .30])

	Z AT θ =								
MARKER	.00	.01	.05	.10	.20	.30	.40	Z_{max}	θ_{max}
Severe or mild (possible) disorder:									
D7S505	3.36	3.33	3.14	2.79	1.90	.99	.31	3.36	.00
eNOS	1.96	2.11	2.42	2.37	1.69	.86	.23	2.44	.06
D7S483	.62	.76	1.12	1.27	1.08	.63	.21	1.05	.14
Severe disorder only:									
D7S505	3.15	3.09	2.78	2.36	1.49	.72	.19	3.15	.00
eNOS	1.80	1.86	1.95	1.83	1.27	.64	.18	1.95	.04
D7S483	.73	.80	.94	.94	.69	.34	.19	.95	.08

= 3.53 at θ = .12. Finally, Z_{max} = 2.96 at θ = .00 was obtained for affecteds-only analysis (q = .1; f = .01).

Nonparametric Linkage Analyses of Nuclear and Multiplex Families

Nonparametric linkage analysis using the TDT test (Terwilliger 1995) and the APM method (Weeks and Lange 1992) was performed with both nuclear and multiplex families and with the analysis restricted to women with severe disease. The former test looks at all affected offspring of heterozygous parents and compares the frequency at which they transmit any allele to their affected offspring with the frequency at which they transmit the other alleles to their affected children. Further support for linkage was obtained for the intragenic marker eNOS(CA)$_n$ (λ_{TDT} = .89; −2ln(L) difference = 6.43; P = .005), whereas results for markers D7S505 and D7S483 did not reach a significant level (λ_{TDT} = .75; −2ln(L) difference = 2.55; P = .055 and λ_{TDT} = .55; −2ln(L) difference = 0.01; P = .47, respectively). The TDT results from both populations were in good agreement, but more of the deviance was attributed to distortion of allele segregation in the Scottish than was seen in the Icelandic data (fig. 1). The TDT results of the eNOS(CA)$_n$ marker showed that the distortion of transmission of allele 14 was most significant in both populations, and again the Scottish data contributed more to the deviance (table 4). When the TDT likelihood differences for the three markers were examined, the maximum distortion was observed for the eNOS(CA)$_n$ marker, rather than for the two flanking markers (fig. 1). The more conservative approach of multilocus analysis, rather than the single-locus module, of the APM program was also used, showing further evidence for distortion, from independent segregation of the marker alleles in these families ($T_{[fp)=sqrt(p)]}$ = 3.53; $P < .001$; and $T_{[fp)=1]}$ = 4.69 ($P < .001$).

Discussion

Many common diseases, such as diabetes, ischemic heart disease, multiple sclerosis, and schizophrenia, are likely to be etiologically and genetically heterogeneous, which means that the same clinical disorder is caused by

Table 3

Two-Point Z Analyses Using D7S505 and Various Genetic Parameters

	Z AT θ =								
MODEL[a]	.00	.01	.05	.10	.20	.30	.40	Z_{max}	q_{max}
1	−∞	−6.01	.24	2.22	2.66	1.69	.56	2.77	.16
2	2.52	2.54	2.48	2.25	1.54	.80	.24	2.54	.02
3	4.03	4.01	3.82	3.43	2.36	1.24	.38	4.03	.00
4	.34	1.12	2.79	3.48	3.11	1.88	.63	3.53	.12
5	2.96	2.94	2.78	2.47	1.68	.89	.29	2.96	.00

[a] 1 = Autosomal recessive with complete penetrance, as suggested by Chesley and Cooper (1986); 2 = autosomal recessive with incomplete penetrance, as suggested by Liston and Kilpatrick (1991); 3 = genetic model with partial dominance, derived from Arngrímsson et al. (1995); 4 = autosomal dominant with 90% penetrance; and 5 = affecteds-only analysis.

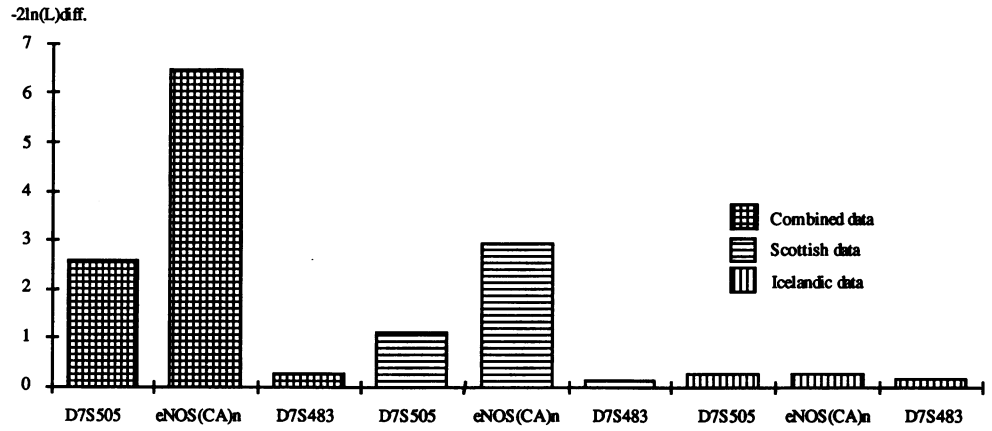

Figure 1 Multiallelic statistic. The TDT likelihood differences (−2ln(L)diff.) for each marker in a 4-cM region around the eNOS gene are shown for combined family data, as well as for Scottish and Icelandic families separately. The maximum-likelihood estimate of λ_{TDT} is .89, .90, and .81 for the combined, Scottish, and Icelandic data, respectively.

different susceptibility genes and environmental factors. This is also likely to be the case with preeclampsia or pregnancy-induced hypertensive syndrome, which arises only during pregnancy. We have tested the hypothesis that the eNOS gene might be involved in the predisposition to familial pregnancy hypertension. Results obtained in this study suggest linkage and are supported by the following arguments. First, the affected-sib-pair results are corroborated by positive findings from the three markers tested in the region of the eNOS gene, which reduces the risk of the type of false-positive result that can be observed with a single marker. Also, no evidence of segregation distortion was observed among affected-nonaffected sib pairs. Second, parametric as well as nonparametric methods confirmed the linkage between the locus and the disease. Third, the Z_{max} for the three markers did not seem to be sensitive to changes in genetic parameters, with significant LOD scores being observed at D7S505 for most genetic models but at different θ values. This effect would be expected in the presence of linkage, if the genetic parameters are not accurately formulated (Durner and Greenberg 1992). New nonparametric methods designed to detect linkage and linkage disequilibrium at a short distance from the genetic aberrations also supported the conclusion that these markers lie within close reach of a molecular aberration predisposing to familial pregnancy-induced hypertension. The eNOS marker did not give the most significant results for linkage by LOD-score calculations; the most significant results were given by marker D7S505 located ~2 cM from the eNOS gene. These results can be interpreted under the following hypothesis, which is compatible with the findings at this stage. The eNOS gene might be one of the genes involved in the development of the familial form of the disease, but possible heterogeneity, existence of phenocopies, or inaccurate specification of parameters in the genetic models might inflate the recombination value. This would falsely position the genetic aberration for the syndrome at some distance from the eNOS gene when the LOD-score method is used but not when a model-free approach is applied. Indeed, the results from the TDT link-

Table 4

TDT Analyses of eNOS $(CA)_n$ Marker in Icelandic, Scottish, and Combined Family Data Classifying Patients with Severe Pregnancy-Induced Hypertensive Disorder as Affected

	Scottish Families				Icelandic Families				All Families			
Allele	No. of Cases	No. of Controls	TDT[a]	P[b]	No. of Cases	No. of Controls	TDT[a]	P[b]	No. of Cases	No. of Controls	TDT[a]	P[b]
10	5	5	.00	.99	5	6	.09	.98	10	11	.05	.999
14	10	1	7.36	.05	6	1	3.57	.17	16	2	10.9	.007
15	4	7	1.92	1.00	4	3	.14	.87	8	10	.02	.999

[a] Estimates for alleles that appear at least five times in each of the two data sets.
[b] Corrected for multiple testing.

age analysis using only women with the more secure diagnosis of severe pregnancy-induced disease shows maximum distortion of independent segregation of the marker alleles at the eNOS microsatellite. It may be of relevance that in both populations the strongest association was found with allele 14 of the eNOS marker. Results obtained with the HOMOG program (Ott 1991) were also consistent with this hypothesis, with ~70% of families being linked to the eNOS locus, but the results were not statistically significant (data not shown). An alternative hypothesis would specify that the eNOS gene is not involved in the pathogenesis of the disease but is in close proximity to the actual genetic aberration on a locus that we have identified by chance, owing to the presence of a candidate gene in the region. A search for reports of other accurately positioned candidate genes in the region of D7S505 was not successful but does not exclude the existence, in this region, of an unknown gene that could only be identified by cloning the genes present in the region.

Several strategies can be envisaged to establish which of these two hypotheses accounts for linkage. Among them is the identification of polymorphisms in the region, which can be used in search for linkage disequilibrium with disease-associated alleles.

To our knowledge, this is the first report to show strong evidence for linkage with the familial pregnancy-induced hypertensive phenotype. Our previous report on the angiotensinogen gene (Arngrímsson et al. 1993), which was also supported by an independent association study (Ward et al. 1993), indicated linkage with only a subset of the 22 families investigated. Although the APM method showed significant results in that study, no significant evidence in favor of linkage was obtained by use of other linkage methods—for example, by LOD-score calculations using several genetic models. In a transgenic hypertensive-animal model, the importance of angiotensinogen has now also been suggested, in which interaction between the maternal angiotensinogen gene and the fetal/placental renin gene is described as necessary and sufficient to predispose the mother toward development of a pregnancy-induced hypertensive-like syndrome (Takimoto et al. 1996). However, in humans not all investigators have observed association or linkage with the angiotensinogen locus (Wilton et al. 1995). This raises the important question of clinical and molecular heterogeneity in this complex disorder; for example, it would be reasonable to ask whether family members in our study are at higher risk of cardiovascular diseases later in life and, furthermore, whether families in which the disease is linked to the eNOS-gene region are indicative of a subset of pregnancy-induced hypertensive disorder with health implications beyond pregnancy, although at the time of delivery no clinical or biochemical differences can be observed between the different subsets. At present, such conclusions cannot be drawn, since we do not have enough information about the later health of the family members, and because the small number of individuals in each age group makes comparison with a population cohort difficult. These questions can only be answered by longer follow-up on the health of these women and of suitable controls. However, if it turns out that a genetic marker will help to define a subset of women who not only are prone to pregnancy-induced hypertensive disorder but also are at greater risk of developing cardiovascular diseases later in life, this would be of fundamental medical importance. It would have implications for the management of these women and facilitate the study of hypertensive traits much earlier in life.

Both angiotensin and NO affect vasoregulation. High levels of angiotensin cause vasoconstriction, whereas NO mediates vasodilatation. During normal pregnancy, systemic adaptation occurs, with physiological changes in the cardiovascular circulation, leading to vasodilatation and decrease in systemic vascular resistance. This adaptation does not occur in severe pregnancy-induced hypertensive disorders, and abnormalities in endothelial cell–related function have been demonstrated (Roberts and Redman 1993). Peripheral resistance is increased (Groenendijk et al. 1984), and there is a greater sensitivity to pressor agents such as angiotensin II (Gant et al. 1973). This has recently been related to a reduction of NO synthesis (Delacretaz et al. 1995). NO produced by eNOS is a principal determinant of the vascular tone (Griffith et al. 1987). Once synthesized, it diffuses from the endothelial cells to smooth-muscle cells, where it induces smooth-muscle-cell relaxation by activation of its second messenger, cyclic guanosine 3′,5′-monophosphate. Several lines of evidence suggest the role of NO in the development of pregnancy-induced hypertension. Pharmacological blockade of NO production in pregnant experimental animals causes changes similar to those in humans (Yallampalli and Garfield 1993; Molnar et al. 1994). Endothelial-dependent vasodilatation has been shown to be impaired in arteries from preeclamptic women (McCarthy et al. 1993), and similar findings have been described in nonpregnant mice lacking the eNOS gene (Huang et al. 1995). Also, sera from affected women are a more potent stimulant of NO production in normal endothelial cell culture than are sera from normotensive women (Baker et al. 1995), indicating some form of a blockage of the NO-production pathway in the endothelium of these women, with activation of a feedback mechanism and accumulation of substances that stimulate NO synthesis. Recent studies show that cAMP production in the placental circulation is reduced in hypertensive pregnancies compared with pregnancies in normotensive women (Kovacs et al. 1994).

The cause of pregnancy-induced hypertension is still unknown, despite decades of intense research into the

pathogenesis of the disease. It is interesting that evidence for linkage was obtained in these families both when the more homogeneous group of women with the severe form of the disorder were classified as affected and when women with mild disease or only transient hypertension in pregnancy were included. Apparently the familial form is characterized by a spectrum in which the severity of the disease can be variable. It may be too early to draw conclusions about the possible role of genes in this chromosomal region in sporadic cases of the disease, and it is still not known whether the familial form represents another category of hypertensive disorders. Identification of different genetic loci at play in pregnancy-induced hypertension will progressively help in the delineation of important components in the development of the trait. By elucidating the mechanism of the familial form, these studies might also help in the understanding of other forms of gestational hypertension, including sporadic cases. In this context the identification of predisposing alleles strongly associated with the disease could be of assistance in the identification of women at high risk of developing the condition and thus could influence the future organization of antenatal care and management of pregnant women.

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