Detection of CAG repeats in pre-eclampsia/eclampsia using the repeat expansion detection method

K.A. Freed1,3, D.W. Cooper2, S.P. Brennecke1 and E.K. Moses1

1Department of Perinatal Medicine, The Royal Women’s Hospital and Department of Obstetrics and Gynaecology, University of Melbourne, Carlton, Victoria, Australia and 2School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, New South Wales, Australia
3To whom correspondence should be addressed at: Pregnancy Research Centre, The Royal Women’s Hospital, 132 Grattan Street, Carlton 3053, Australia. E-mail: katy.freed@rwh.org.au

Pre-eclampsia/eclampsia is a serious disorder of human pregnancy that has a worldwide incidence of 2–10% and carries a severe morbidity and mortality risk for both mother and child. Its precise cause remains unknown. However, there is increasing evidence of an underlying complex maternal genetic susceptibility. Its high population incidence in the face of strong negative selection pressure suggests that the gene(s) involved have a selective advantage and/or a high mutation rate. One class of genetic diseases that involves high mutation rate are the trinucleotide repeat expansion diseases. Thus, the aim of this study was to determine whether there is an association between a trinucleotide (CAG) repeat expansion and pre-eclampsia/eclampsia. We have used the repeat expansion detection (RED) method, which was developed to directly identify clinically significant repeat expansions, to analyse genomic DNA from an Australian and New Zealand population. The maximal CAG repeat length for each individual was recorded and the Mann–Whitney U and Wilcoxon rank sum test for independent samples were used to compare distributions for CAG/CTG repeats between two populations. There were no statistically significant differences between the distribution of CAG repeats in normotensive (n = 59) and severe pre-eclampsia (n = 69) (Mann–Whitney U = 1732; P = 0.14), and normotensive (n = 59) and eclamptic (n = 15) populations (Mann–Whitney U = 417, P = 0.726). Therefore, these RED results do not support a role for a large CAG expansion in pre-eclampsia/eclampsia. However, these data do not preclude the possibility that a small CAG expansion is associated with the disorder nor do they negate the hypothesis that a highly mutable gene contributes to the genetic component of pre-eclampsia/eclampsia.

Key words: CAG repeats/genetics/pre-eclampsia

Introduction

The pre-eclampsia/eclampsia syndrome is one of the most dangerous complications of human pregnancy. It has a worldwide pregnancy incidence of 2–10% and carries a severe morbidity and mortality risk for both mother and child. Pre-eclampsia/eclampsia is unique to human pregnancy and has been identified in all human ethnic groups for which data exist. Occurring in the mid-to-late stages of pregnancy, pre-eclampsia is traditionally diagnosed by three clinical signs, pregnancy-induced hypertension and proteinuria, and generalized oedema. Untreated, severe clinical deterioration is marked by maternal multisystem organ dysfunction and fetal compromise. The added complication of grand-mal convulsions distinguishes eclampsia from pre-eclampsia (Roberts and Redman, 1993; Working Group on High Blood Pressure in Pregnancy, 2000). Currently, the only effective amelioration of the symptoms is delivery of the baby and placenta, irrespective of gestation.

Eclampsia has been recognized since ancient times (Chesley, 1974). Yet, the pre-eclampsia/eclampsia syndrome is still described as a ‘disease of theories’ (Higgins and Brennecke, 1998). The proposed list of contributing aetiological factors include immunological, placental and endothelial factors (Roberts and Redman, 1993; Ness and Roberts, 1996; Dekker and Sibai, 1998; Redman et al., 1999). Accumulating evidence suggests that there is a genetic predisposition to develop pre-eclampsia/eclampsia, illustrated by family studies showing that the incidence of pre-eclampsia/eclampsia in mothers, daughters, sisters and grand-daughters of probands is two to five times higher than in mothers-in-law, daughters-in-law and other controls (Chesley et al., 1968; Sutherland et al., 1981; Chesley and Cooper, 1986; Arngrimsson et al., 1990). More recently, a large study has shown pre-eclampsia heritability to be 0.54 in a Swedish population (Salonen Ros et al., 2000).

The mode of inheritance that best fits pre-eclampsia/eclampsia continues to be the subject of extensive debate. A number of segregation analyses have attempted to fit the recurrence risk data from the family studies to a genetic model, but as yet there is no universal agreement. Early reports suggested a recessive gene hypothesis (Cooper and Liston, 1979; Liston and Kilpatrick, 1991) or a model of dominant inheritance with incomplete penetrance (Arngrimsson et al., 1990). More recently, other authors have proposed a maternal and fetal genotype interaction (Lie et al., 1998; Esplin et al., 2001).

Two fascinating and rarely asked questions are (i) how/why did the pre-eclampsia/eclampsia syndrome evolve and (ii) how has it remained in the global population since antiquity, given the potential maternal and fetal lethality in first partner pregnancies. Haig (1993) proposed that, central to any dialogue on fetal survival during pregnancy, one should consider the concept of maternal/fetal conflict.
According to the genetic conflict theory, instead of a co-operative interaction between a mother and her fetus, natural selection forces dictate that there will be genetic conflict between maternal and fetal genes. Hence, fetal genes will be selected to increase nutrient transfer to the fetus and maternal genes will be selected to limit transfer in excess of some maternal optimum. Thus, Haig’s conflict hypothesis predicts that fetal genes will enhance maternal blood flow through the intervillous space by decreasing resistance in the utero-placental circulation and/or increasing resistance in the non-placental maternal circulation (Haig, 1993).

An alternative hypothesis proposed by one of us (D.W.Coop) is that gene(s) that confer susceptibility to pre-eclampsia/eclampsia may be highly mutable, thus maintaining the disorder in the population. One type of highly mutable genetic variation was first discovered independently by investigations into the mutations underlying fragile X syndrome (Fu et al., 1991; Kremer et al., 1991; Verkerk et al., 1991), a relatively common mental retardation disorder, and spinobulbar muscular atrophy (LaSpada et al., 1991), a rare neurodegenerative disorder. In both cases, the molecular event occurred in a DNA region containing trinucleotide repeats. A new form of mutagenesis was discovered and termed ‘dynamic mutation’ because of the highly mutable nature of the DNA segments involved (Richards and Sutherland, 1992). Dynamic mutation has been found to have a fundamental role in human genetics, accounting for what appeared to be non-Mendelian patterns of inheritance. Since 1991, 16 human neurological and neuromuscular genetic disorders, including fragile X (Kremer et al., 1991; Verkerk et al., 1991) and Huntington disease (The Huntington’s Disease Collaborative Research Group, 1993), have been identified in which a normally polymorphic trinucleotide repeat undergoes a mutational change, whereby the repeat length expands, sometimes quite dramatically, resulting in altered gene function (Jasinska et al., 2003). Therefore, the discovery of trinucleotide repeat expansion mutations has uncovered a previously unrecognized form of mutational change and has provided a molecular explanation for some unusual inheritance patterns. Genetic disorders outside the central nervous system, such as retinitis pigmentosa (Keen et al., 1997) and familial total anomalous pulmonary venous return (Bleyl et al., 1995), have been investigated as candidates for repeat expansion mutation diseases. Recent studies demonstrating an association of repeat number with cancer (Ferro et al., 2002) and male infertility (Dowsing et al., 1999) suggest that variation in repeat copy number may have an even more general and fundamental role in molecular genetics (Ferro et al., 2001). Therefore, the highly mutable nature of the repeats involved may explain the continued existence of pre-eclampsia/eclampsia in the population in the face of strong negative selective pressure.

There are a number of features of repeat expansion mutation that are applicable to pre-eclampsia. All the expansion diseases have been shown to be inherited. Along with pre-eclampsia, the dynamic mutation diseases display inheritance patterns that do not conform to traditional Mendelian segregation. The meiotic instability of the repeat tract, which leads to intergenerational differences, allows progression through a pedigree to be monitored. This change in repeat number, primarily an expansion, is the underlying factor in one of the features of dynamic mutation, anticipation. Anticipation is the occurrence of increased severity of the disease and/or earlier onset of disease with increasing number of repeats over generations (Petronis and Kennedy, 1995). Determining whether anticipation occurs in pre-eclampsia/eclampsia offers particular challenges. As pre-eclampsia/eclampsia is a reproductive disorder, correlating age of disease onset with number of repeats may be inconclusive. In relation to severity of disease, the level of medical intervention, rather than the underlying pathophysiology, may be the determining factor as to whether a woman experiences mild pre-eclampsia, severe pre-eclampsia or eclampsia.

One of the striking features of trinucleotide expansion is that, in addition to meiotic instability, some disorders also display mitotic instability, which results in somatic mosaicism. Tissue-specific variability has been reported in Huntington disease. The target tissue/cell exhibits increased copy number compared with other tissues/cells (De Rooij et al., 1995; Kennedy and Shelbourne, 2000). In the case of Huntington disease, the expanded repeat leads to selected neuronal death in the brain. Therefore, this type of tissue-specific instability could occur in pre-eclampsia resulting in only certain tissues, such as the placenta and/or decidua, being affected. The potential role of apoptosis in the toxic gain-of-function in the polyglutamine diseases correlates with evidence that apoptosis in placental tissue may play a role in pre-eclampsia (DiFederico et al., 1999; Ishihara et al., 2002; Crocker et al., 2003; Pang and Xing, 2004).

The majority of repeat expansion diseases are caused by a CAG expansion, in particular, those that are located in the coding region which are translated into polyglutamine stretches. Several different strategies have been used to search for CAG repeat expansions in association with disease including: (i) direct analysis of genomic trinucleotide repeats to identify large expansions; and (ii) pedigree studies of candidate genes that harbour repeated trinucleotide stretches. The most widely used method for directly identifying clinically significant CAG repeat expansions, termed repeat expansion detection (RED), was developed by Schalling et al. in 1993. RED provides a direct method to determine whether repeat expansions are associated with human genetic disease. It uses the principle that long trinucleotide repeats in genomic DNA are able to serve as a template to catalyse the ligation of oligonucleotides into larger multimers, which reflect the size of the largest trinucleotide repeat located anywhere in the genome. The size of the largest ligation product is the result recorded for each individual. A comparison between the distribution of maximal repeat lengths from individuals in a control population and from individuals in a disease population is then made. The advantage of this technique is that neither prior knowledge of chromosomal location nor flanking sequence information is required.

The RED assay to detect CAG repeats has been used successfully in a number of studies (Bleyl et al., 1995; Morris et al., 1995; O’Donovan et al., 1995; Keen et al., 1997), including spastic paraplegia (Nielsen et al., 1997), testicular cancer (King et al., 1997) and autosomal dominant cerebellar ataxia patients not resulting from known genes (Pujana et al., 1998) and has been found to be a reliable and highly reproducible method (Hofferbert et al., 1997; Martorell et al., 1997; Sirugo et al., 1997). The hypothesis tested in this study is that a CAG repeat expansion contributes to the genetic component of pre-eclampsia/eclampsia. As the RED assay provides a direct method to determine whether repeat expansions are associated with human genetic disease, the aim of this study was to use the RED assay to ascertain whether there is a genomic CAG repeat expansion in association with pre-eclampsia/eclampsia.

Materials and methods

Samples

The samples used in the RED population study were (i) DNA extracted from peripheral blood collected from women presenting at the Royal Women’s Hospital, Melbourne, Australia (normotensive pregnant controls, n = 28; severe preeclampsia, n = 4) and (ii) DNA from female members of pre-eclampsia/eclampsia pedigrees including 28 Australian and New Zealand families (normotensive pregnant controls, n = 31; severe pre-eclampsia, n = 65; eclampsia, n = 15). Details of the predominantly Caucasian subjects of the pre-eclampsia/eclampsia pedigrees have been presented in previous investigations into the genetic basis of pre-eclampsia/eclampsia (Wilton et al., 1990; Humphrey et al., 1995; Harrison et al., 1997; Guo et al., 1999; Lade et al., 1999; Moses et al., 2000; Kaiser et al., 2004). Pre-eclampsia and eclampsia were defined.
according to the guidelines set out by the Australasian Society for the Study of Hypertension in Pregnancy consensus statement (Brown et al., 1993). Severe pre-eclampsia was defined as maternal hypertension which developed in late pregnancy (after 20 weeks of gestation) and resolved within 3 months post-partum, in association with significant proteinuria (>300 mg/24 h). Maternal hypertension was defined as (i) the presence of at least 140 mmHg of systolic pressure and/or a diastolic pressure of at least 90 mmHg or (ii) a rise from baseline in systolic blood pressure of at least 30 mmHg and/or a rise from baseline of at least 15 mmHg of diastolic blood pressure. These levels occurred on at least two occasions, 6 h or more apart. The level of proteinuria was at least 300 mg in a 24 h specimen or at least 1 g/l (± on dipstick) in a random urine collection. Other clinical signs that contributed to the diagnosis of pre-eclampsia included generalized oedema, low platelet count and elevated serum urate levels. Women who met these criteria and experienced convulsions or unconsciousness in the perinatal period were diagnosed as having eclampsia. Other causes of hypertension such as kidney disease and essential hypertension and autoimmune disease were excluded in the diagnosis of pre-eclampsia.

The work described in this study was approved by The Royal Women’s Hospital Research and Ethics Committees. Written, informed consent was obtained from all participating patients by a clinical research midwife before the samples were collected.

Peripheral venous whole blood (>3 10 ml/individual) was collected into S-Monovette® EDTA–coated blood tubes (Sarstest, Numbrecht, Germany) and stored frozen at –40°C before extraction. Genomic DNA was isolated from 10 ml of whole blood (thawed on ice) using a commercial genomic DNA extraction system, QIAGEN blood and cell culture midi kit (QIAGEN, Hilden, Germany). All steps pertaining to the genomic DNA extraction were carried out as specified in the QIAGEN genomic DNA extraction protocol. The extracted genomic DNA was stored at 4°C in 1.5 ml microfuge tubes (Elskay, Shrewsbury, MA, USA) until use.

In order to determine the sensitivity of the RED method, genomic DNA was analysed from one patient with Huntington disease (42 CAG repeats) for which the number of CAG repeats for the disease locus had been determined using standard PCR-based methods. In addition, a DNA sample from the Australian/New Zealand population study was chosen as an internal control that was included in all RED assays.

**RED assay**

The RED assay was performed according to the method of Schalling et al. (1993), with some minor modifications. In order to improve the sensitivity and reliability of the assay, Zander et al. (1998) used multivariate optimization analysis to assess the variables that exert the greatest influence on the RED reaction. On the basis of these findings, a number of pilot experiments were carried out to optimize the RED conditions, including testing variations in the length/type of oligonucleotide, oligonucleotide-purification methods, annealing/ligation temperature, amount of genomic DNA, amount of oligonucleotide in RED reaction, number of cycles and amount of thermostable ligase (data not shown). To test the reproducibility of the optimized RED conditions, two RED reactions were undertaken, 1 month apart, using one DNA sample that had been stored at 4°C. The maximal repeat length was 64 repeats, which was detected in both RED assays (data not shown). The final RED conditions used for the population study are described below.

**Oligonucleotide purification and phosphorylation**

Before the RED ligation reaction, the (CAG)₉ oligonucleotides to be used in the RED procedure were gel purified and then phosphorylated at the 5’ end using T4 polynucleotide kinase (PNK). Gel purification of oligonucleotides used in the RED procedure is crucial in obtaining efficient and reliable ligation. The presence of incomplete synthesis products can result in very low ligation efficiency and ligation artefacts (Sirugo and Kidd, 1995). Firstly, (CAG)₉ oligonucleotides (Micronon, Monash University, Melbourne, Australia) were resuspended in MilliQ H₂O. Equal volumes of crude oligonucleotide and loading buffer [40% (v/v) glycerol, x1 TBE] were mixed, loaded onto a 15% polyacrylamide (3 M urea) gel situated in a Hoefer tank (SE 250-Mighty Small II slab gel electrophoresis unit; Hoefer Scientific Instruments, San Francisco, CA, USA) and then electrophoresed at 50 W for 12 min. A dye mixtare [40% (v/v) glycerol, x1 TBE, 0.05% (w/v) bromophenol blue/xylene cyanol] loaded in the wells flanking the oligonucleotides was used as a marker; on a 15% polyacrylamide gel, xylene cyanol migrates at approximately 30 bp, whereas bromophenol blue migrates at approximately 10 bp (Sambrook and Russell, 2001).

Following electrophoresis, the (CAG)₉ oligonucleotides were visualized on the gel by UV shadowing (Sirugo and Kidd, 1995). The gel was placed on top of a fluorescent thin-layer chromatography (TLC) plate (POLYGRAM® SIL N-HR/UV254, 0.2 mm silica gel TLC plate; Macherey-Nagel, Düren, Germany), a portable short wavelength UV lamp held directly over the gel, and the predominate DNA ‘shadows’ lying at approximately 24 bp between the dye markers were cut out with a sterile scalpel blade. These gel slices were placed into 1.5 ml microcentrifuge tubes and then finely chopped with a sterile scalpel blade before the addition of 1 ml of TE, pH 7.4. The tube was incubated at 55°C overnight in a heat block to allow the oligonucleotides to diffuse from the gel into the TE buffer.

The oligonucleotides were then desalted by passing the solution through a Sep-Pac® C₈ cartridge (Waters Corporation, Milford, MA, USA) (Sambrook and Russell, 2001), eluted with 1 ml of a 60:40 (v/v) mixture of methanol: H₂O, and a spectrophotometric reading at 260 nm was taken before the solution was dried in an RC 10.22 centrifugal evaporator (Juran, Saint-Herblain, France). The dried pellet was resuspended in MilliQ H₂O and stored at –20°C.

**RED reaction conditions**

RED ligation reactions (10 µl) containing 4 µg of genomic DNA, 1.5 pmol of phosphorylated gel-purified oligonucleotide, 15 U of Ampligase®-thermostable DNA ligase (Epitrent, Madison, WI, USA) and 1 µl of the supplied 10 Ampligase® buffer were assembled in a 0.2 ml PCR tube (MicroAmp® reaction tube with cap) (PerkinElmer, Wellesley, MA, USA). Samples were heated for 5 min at 95°C, then subjected to 450 cycles of 94°C for 10 s and 64°C for 30 s in a GeneAmp PCR system 9700 (PerkinElmer). The RED products were size separated on a 6% 16 M polyacrylamide–sequencing gel by electrophoresis.

**Electrophoresis and detection of RED products**

Two microlitres of RED dye (95% (v/v) formamide, 20 mM EDTA, pH 8.0, 0.03% (w/v) bromophenol blue/xylene cyanol) was added to the RED reaction, mixed, heated to 95°C for 8 min to denature and concentrate the samples and placed on ice before loading. The wells of the gel were flushed with x1 TBE and then the samples (2 µl) were loaded in alternate lanes to avoid any cross-contamination between lanes. Electrophoresis of the reactions was performed for 55 min at 70 W constant power until the xylene cyanole dye front had migrated approximately 20 cm. The DNA was transferred from the polyacrylamide gel onto Hybond N+ positively charged nylon membrane (Amersham Biosciences, Buckinghamshire, UK) by capillary action overnight. After transfer, the filter was peeled off the gel and briefly washed with x6 SSC to remove adhering gel. The filter was placed on blotting paper and dried at 80°C for 10 min. The DNA was UV cross-linked to the filter for 5 min using a UV m-40 MightyBright UV transilluminator (Hoefer Scientific Instruments, San Francisco, CA, USA).

To visualize the RED products, the filter was probed with a radiolabelled (CTG)₁₀ oligonucleotide. Firstly, the filter was prehybridized at 42°C for 2 h in 5 ml of hybridization buffer [50% (v/v) formamide, 5× SSPE, 5× Denhardt’s Solution, 0.5% (w/v) SDS, 0.1 mg/ml denatured salmon sperm DNA]. Hybridization was carried out overnight at 42°C in 5 ml of fresh hybridization buffer containing radiolabelled (CTG)₁₀ that had been 3’ end labelled by the addition of [α-³²P]dATP using terminal deoxynucleotidyl transferase (Promega Corporation, Madison, WI, USA). Following hybridization, the filters were washed twice for 10 min in x1 SSC and 0.1% SDS at room temperature, then twice in x0.1 SSC and 0.1% SDS for 20 min at 42°C, and finally autoradiographed at –80°C for 1–4 days on Agfa X-ray film using two intensifying screens. The maximal size of each ligation product for each sample was scored directly from the autoradiograph.

**Statistical analysis**

The Mann–Whitney U and Wilcoxon rank sum test for independent samples were used to compare distributions for CAG/CTG repeats between two populations.
The two populations used in this study were (i) women who had reproduced and had an uncomplicated pregnancy and (ii) women who had been diagnosed with either pre-eclampsia or eclampsia. The computations were performed with SPSS software, version 8.0 (SPSS, Chicago, IL, USA).

Results
The autoradiograph presented in Figure 1 was used to determine the sensitivity of the optimized RED method when using a (CAG)_8 oligonucleotide and 1 µg of genomic DNA extracted from a Huntington disease (42 CAG repeats) patient. The maximal CAG repeat tract identified in the Huntington disease DNA was 64 repeats. These data confirm that the RED assay is sensitive enough to detect the CAG repeat tract within the disease locus. The Huntington disease result also shows that the CAG repeat tract within a disease locus within one individual may not be the longest repeat tract in that genome. Thus, to identify clinically relevant repeat tracts, a comparison of the distribution of maximal repeat lengths between two populations is required. Therefore, to ascertain whether a CAG repeat expansion is associated with pre-eclampsia/eclampsia, a population study using RED analysis was performed.

A representative autoradiograph from the RED population study is presented in Figure 2. The data generated from the RED assay comparing the maximal CAG repeat length between severe pre-eclamptic (n = 69) and normotensive (n = 59) DNA are presented in Figure 3. The distribution of maximal CAG repeat lengths between the two populations was not statistically significant (Mann–Whitney U = 1732; P = 0.14). The distribution of maximal CAG repeat lengths between eclamptic (n = 15) and normotensive (n = 59) DNA samples (Figure 4) was also not significant (Mann–Whitney U = 417; P = 0.726).

Discussion
In this study, the RED assay was used to compare the distribution of maximal CAG repeat lengths between normal and pre-eclamptic/eclamptic samples. There were no statistically significant differences detected between either normal and pre-eclamptic or normal and eclamptic groups. These data suggest that a large CAG expansion is not associated with pre-eclampsia/eclampsia. While CAG repeat loci were the focus of this study, the putative role of other disease-associated triplets [CGG (fragile X), GCC (fragile XE), CTG (myotonic dystrophy) and GAA (Friedrich ataxia) (Cummings and Zoghbi, 2000)] have yet to be investigated. Therefore, these data do not negate the hypothesis that a highly mutable gene contributes to the genetic component of pre-eclampsia/eclampsia, nor do they exclude a role for other expanded repeats contributing to the disorder.
Although a large clinically associated CAG expansion could not be detected, the RED results do not preclude the possibility that a small CAG repeat expansion, such as that seen in spinocerebellar ataxia 6 (non-disease 4–16 repeats, ataxia 21–27 repeats), (Zhuchenko et al., 1997), may be associated with pre-eclampsia/eclampsia. One of the main disadvantages of the RED assay is the inability to detect small expansions. Non-disease-related expansions occur frequently in the population (Lindblad et al., 1995; Sirugo et al., 1997), which complicate the interpretation of disease-association studies. In this study, 52% of normal controls screened had maximal repeat sizes of 64 repeats (192 bp) or greater. Schalling et al. (1993) and Lindblad et al. (1995) described expansions above 60 CAG repeats in about 30% of normal European populations. The high number of small expansions detected in the background population or control sample limits the ability of the RED technique to detect smaller repeat expansions associated with disease; therefore, trinucleotide-repeat expansions within the size ranges found in Kennedy disease (40–42 repeats), dentatorubral-pallidolysian atrophy (54–70 repeats) and spinocerebellar ataxia 1 (41–81 repeats), could potentially be missed by this screening method.

Variation in repeat numbers has been investigated in different global populations. Sirugo et al. (1997) used RED analysis to investigate the distribution of CAG maximal repeat lengths in human and non-human primates. They found that a large proportion of the Chinese, Japanese, Rondonian Surui, Maya and Mbuti/Biaka Pygmy populations have moderately long (50–70) CAG repeats. The distribution of maximal CAG repeat lengths in the normal Australian/New Zealand population presented in this study (primarily Northern European/Anglo-Saxon descent) also shows bias towards moderately long repeats. In two comprehensive studies of the global variation of polymorphic loci associated with disease, Richards et al. (1996) and Andres et al. (2002) concluded that population differences account for a small part of the total variation found, and most of the diversity is determined by the characteristics of each locus.

**Figure 3.** The distribution (frequency) of the maximum number of CAG repeats in normal (n = 59) and pre-eclamptic (n = 69) populations as determined by repeat expansion detection. No statistically significant (P = 0.14) distribution change in association with pre-eclampsia was identified.

**Figure 4.** The distribution (frequency) of the maximum number of CAG repeats in normal (n = 59) and eclamptic (n = 15) populations as determined by repeat expansion detection. No statistically significant (P = 0.726) distribution change in association with eclampsia was identified.
As RED is a multilocus assay, it has not yet been established whether this technique could detect expansions for a complex disorder with locus heterogeneity and possible involvement of numerous susceptibility genes. It has been shown that CAG RED is capable of detecting small expansions against a background of polymorphic larger repeat sizes as long as the disease population is homogeneous (Hofferbert et al., 1997). In genetically heterogeneous disorders, a relatively minor contribution of a trinucleotide-repeat expansion at a single locus could be difficult to detect. Despite the limitations of the RED assay, it has been used successfully in a number of association studies (Morris et al., 1995; O’Donovan et al., 1995; Nielsen et al., 1997; Pujana et al., 1998). In addition, Sirugo and Kidd (1998) have utilized the RED method to ascertain maximum length and relative stability of uninterrupted telomeric (TTAGGG) arrays, both in vitro and in vivo. RED has also been adapted and incorporated into a more complex technique in an effort to find expanded alleles in association with disease (Koob et al., 1998).

In conclusion, results generated using the RED method in this study do not support a role for a large CAG expansion in pre-eclampsia/ eclampsia. However, these data do not preclude the possibility that a small CAG expansion is associated with the disorder, nor does it negate the hypothesis that a highly mutable gene contributes to the genetic component of pre-eclampsia/eclampsia. Therefore, in the current genetic studies that are aimed at identifying pre-eclampsia/ eclampsia susceptibility genes, it may be expedient to fully characterize any candidate genes that contain repeated motifs. To address the potential role of small CAG expansions in association with disease, various groups have approached the problem by identifying cDNAs that contain CAG repeats and then using these candidates in case-control/ pedigree studies to find an expansion in association with disease. This approach has been successful in the identification of disease genes including the MJD gene (Kawaguchi et al., 1994). For the exclusion of an aetiologic role for CAG repeat expansion in pre-eclampsia/ eclampsia, testing of individual genes that contain trinucleotide repeats is necessary. This is currently being addressed in our laboratory by characterizing CAG repeat-containing genes expressed in human placenta and decidua.

Acknowledgements

We gratefully acknowledge the support of the research midwives and patients who contributed to this study. We also wish to thank Dr Susan Forrest for her advice on trinucleotide repeat diseases and for providing the repeat expansion disease genomic DNA. This work was supported by the Royal Australian and New Zealand College of Obstetrics and Gynaecology Glyn White Fellowship and a Royal Women’s Hospital 3AW research grant.

References


Submitted on May 6, 2005; accepted on May 13, 2005

Detection of CAG repeats in pre-eclampsia/eclampsia