Towards Genetic Testing in Antenatal Care

Doctoral dissertation

To be presented by permission of the Faculty of Medicine of the University of Kuopio for public examination in Auditorium of Kuopio University Hospital, on Friday 15th March 2002, at 12 noon

Department of Obstetrics and Gynecology
University of Kuopio
Gene tests are becoming an increasingly important part of the health care system in the Western World. In this study we explored the possibility of integrating genetic testing into antenatal care. The work involved fragile X syndrome (FraX) and three disorders belonging to the "Finnish disease heritage": infantile neuronal ceroid lipofuscinosis (INCL), aspartylglucosaminuria (AGU) and congenital nephrosis (CNF), all four causing major postnatal morbidity. AGU and INCL are autosomal recessive diseases causing mental retardation and premature death, whereas CNF is a severe autosomal recessive kidney disease. Fragile X syndrome is a common X-linked cause of mental retardation. There is no cure for the diseases investigated. In this study the feasibility and acceptance of antenatal screening for AGU, INCL, CNF and FraX were explored in maternity care units in Eastern Finland. Furthermore, pregnancy outcomes in FraX carrier mothers were explored. Individuals participating in this study were pregnant women in the area of Kuopio University Hospital in the 1990s. Routine molecular biology techniques, including PCR, Southern Blots and minisequencing, which are currently available for clinical practice, were used in the study.

The acceptance rate of genetic testing was approximately 90% throughout the scope of the diseases tested. Direct mutation analysis involved fewer invasive procedures per affected fetus than conventional screening methods such as trisomy screening. There were no false negative screening results during the study period, and pregnant women found it easy to undergo the screening process.

It can be concluded that it is possible and reasonable to screen for these genetic diseases in an antenatal setting. As the possibilities for genetic testing become broader in the future, open public discussion will be needed to decide which diseases are worth screening for. Accordingly, counseling and legislation concerning genetic testing should be further developed.
ACKNOWLEDGEMENTS

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Finally, I wish to thank my parents for all their love and support.

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Kuopio, February 2002

Juuso Kallinen
**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AFP</td>
<td>alphafetoprotein</td>
</tr>
<tr>
<td>AGA</td>
<td>aspartylglucosaminidase</td>
</tr>
<tr>
<td>AGU</td>
<td>aspartylglucosaminuria</td>
</tr>
<tr>
<td>CGG</td>
<td>cytosine-guanine-guanine trinucleotide</td>
</tr>
<tr>
<td>CVS</td>
<td>chorion villus sampling</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>FraX</td>
<td>fragile X syndrome</td>
</tr>
<tr>
<td>GA</td>
<td>glycoasparaginase</td>
</tr>
<tr>
<td>INCL</td>
<td>infantile neuronal ceroid lipofuscinosis</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>RFLP</td>
<td>restriction fragment length polymorphism</td>
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A. INTRODUCTION

A serious congenital defect or genetic disease threatens the lives of 2 - 3 percent of newborn infants (EUROCAT report 7, Contribution of birth defects to infant mortality - United States, 1986). In the USA congenital anomalies accounted for approximately 22 percent of deaths during the first year of life in 1995 - 1997 (WHO mortality database). The percentage of cases of serious morbidity in infancy and childhood is even higher. The family tragedy is immeasurable. With the growing recognition of the frequency and importance of congenital disorders and with current social trends toward smaller families and delays in childbearing, prenatal diagnosis has an increasingly important role in the management of many pregnancies.

Prenatal diagnosis has usually meant diagnosis of chromosomal disorders. Nowadays, due to progress in gene technology, it is possible to have a much more comprehensive diagnosis. The precondition is that first we must discover the healthy pregnant women without an affected child, who are at risk. The genetic screening of carriers of many severe hereditary diseases is nowadays possible due to the progress that is being made in mapping the human genome and discoveries of gene defects. When using genetic testing in antenatal clinics for detection of severe hereditary diseases, we do not have to use amniocentesis as a screening method, and its use, or that of chorion villus sampling (CVS), only involves mothers whose risk of having an affected child is not only statistical, but proven.

In this study we investigated how genetic screening could be incorporated into antenatal care, and how gene tests could be used as a means of offering more comprehensive prenatal diagnosis to pregnant women. We investigated genetic screening as regards fragile X syndrome (FraX), and three other diseases – aspartylglucosaminuria (AGU), infantile neuronal ceroid lipofuscinosis (INCL) and congenital nephrosis (CNF). These diseases were chosen because FraX is the second greatest cause of mental retardation throughout the world; CNF had been already been screened in the Kuopio region conventionally by measuring AFP; and INCL, AGU and CNF are all members of the so-called Finnish disease heritage. In addition, the availability of high quality DNA diagnoses of these four diseases was essential. Further, we investigated the nature of the risk that a full mutation- or premutation-carrier woman has in her pregnancy and whether a carrier status of
FraX in a woman means a risk of complicated pregnancy and delivery. This study was designed because previous findings suggested that there is, for example, early menopause in FraX premutation carriers, thus possibly indicating problems concerning pregnancy (Schwartz et al. 1994; Partington et al. 1996).
B. REVIEW OF THE LITERATURE

1. GENETIC SCREENING

1.1 History

Prenatal diagnosis originated with the culture and karyotyping of amniotic fluid cells by Steele, Breg and Klinger in 1966 (Steele et al. 1966). It became standard practice to offer prenatal cytogenetic diagnosis to all women of 35 years of age or more (National institute of Child Health, 1975). Hook et al. (1981) showed there to be an increasing frequency of chromosomal abnormalities with advancing maternal age. Low levels of maternal serum alpha-fetoprotein and unconjugated estriol are associated with trisomy 21 and trisomy 18 (Merkaz et al. 1984). Combined use of concentrations of maternal serum human chorionic gonadotropin, unconjugated estriol, and alpha-fetoprotein, and maternal age, can identify approximately 60 percent of cases of Down’s syndrome, with a false positive rate of 6.6 percent (Canick et al. 1988). In fetal-maternal medicine it is widely accepted that 30 - 50 amniocentesis must be carried out to find one case of Down’s syndrome pregnancy (Haddow et al. 1992; Salonen et al. 1997; Heikkilä et al. 1997).

Diagnosing monogenic diseases by means of conventional biochemical tests, such as measurement of enzyme activities and metabolites or the characterization of structurally altered proteins, is now being supplemented by techniques based on DNA amplification. In many cases molecular techniques enable specific, predictive diagnosis even before any phenotypic signs of the disease can be detected. They allow prenatal and preimplantation diagnosis of in-vitro-fertilized embryos, and identification of asymptomatic disease carriers (Handyside et al. 1989; Wagener et al. 1994; Xu et al. 1999; Verlinsky et al. 2001).

Gene frequencies differ among population groups defined on the basis of geography or ethnic and racial background. In different populations various genetic screening programs are used to detect carriers of hereditary diseases. Screening programs to detect thalassemia carriers have been employed in several Mediterranean countries with a high prevalence of the disease (Fessas 1987; Cao and Rosatelli 1993; Curuk et al. 2001). Sickle cell anemia carrier prevalence among
people of African origin is remarkably high, and therefore there are programs to screen for sickle cell anemia carriers, for example in the United States and in the United Kingdom (Davies and Hewitt 1984; Shickle and May 1989; Lorey et al. 1996). Among Ashkenazi Jews there are now nine severe or markedly debilitating autosomal recessive diseases that can be avoided by carrier screening, genetic counselling and prenatal diagnosis (Zinberg et al. 2001). The disease "menu" available for genetic carrier screening is rapidly enlarging due the continuing growth of genetic technology (Edelman et al. 2001; Bargal et al. 2001).

As a public policy, there is currently no antenatal genetic screening in Finland. However, affected families with an index case having a genetic disorder are eligible for genetic counseling and invasive prenatal diagnosis, when appropriate. It is obvious that maternity care plays an important role in this kind of cascade screening and in the counseling process.

1.2 Methods for molecular diagnosis

By sequencing genomic DNA or DNA amplified by the polymerase chain reaction (PCR) it is possible to analyze all types of sequence variants. Sequencing of target DNA is usually considered to be the golden standard for the mutation detection (Nollau et al. 1997). On a large scale and as a routine technique sequencing is impractical and therefore more convenient molecular techniques have been developed. These PCR-based diagnostic methods include allele-specific amplification, allele specific hybridization, oligonucleotide ligation assay (PCR-OLA), restriction fragment length polymorphism (PCR-RFLP), and solid phase minisequencing (Cotton 1993; Ferrari et al. 1996; Nollau et al. 1997). These methods can be used to identify individuals who are not carrying a defective mutation, heterozygous carriers of a mutation, and individuals who are homozygous for the mutation of interest (Romppanen 2000).

In as yet unpublished work by Pastinen et al. (2001) "the Finnish chip" is introduced. This is a deoxiribonucleic acid (DNA) array which is designed to detect most known recessive disease mutations enriched in Finland and belonging to the "Finnish disease heritage", and another 10 known Caucasian mutations, i.e. a total of 31 mutations underlying 27 different clinical phenotypes.
1.3 When to screen? - prospects for antenatal screening

In 1968 the World Health Organization (WHO) published criteria for screening (Wilson and Jungner 1968). These criteria are still valid. Appropriate information, voluntariness, the possibility to discontinue the screening at any of its phases, and quality control, are nowadays also included in a good genetic screening protocol.

WILSON & JUNGRER CRITERIA

1. The condition sought should be an important public health problem.

2. The natural history of the condition should be understood. The disease screened should have a recognizable latent or early symptomatic stage.

3. The screening test should be highly sensitive and specific. The test should also be ethically acceptable.

4. There should be an accepted treatment for patients with recognized disease and this treatment at an early, latent or presymptomatic stage should favorably influence prognosis.

5. Facilities for diagnosis and treatment should be available.

6. The benefit of screening should justify the costs.

7. Case finding should be a continuous process

Programs must conform, first of all, to the requirements and constitutions of common law, and statutory provisions. Moral considerations must also be taken into account. Beneficence, autonomy, and social justice are the three broad principles guiding these considerations. None of these principles can be seen as consistently more important than the others, but the degree to which they are satisfied must be balanced in every instance (Faden et al. 1991). As a general principle, the least burdensome approach, from a legal and ethical viewpoint, that meets public health goals should always be preferred.

Screening is usually considered when all aspects mentioned above are fulfilled. In antenatal screening or prenatal diagnosis the incidence of an abnormality does not correlate with birth prevalence, since not all fetuses survive to term. Antenatal
screening leading to selective abortion also raises ethical issues, which has been the case from the start with Down’s syndrome screening. Such issues have now reached new areas as the molecular techniques have broadened diagnostic possibilities.

1.4 Patient counselling

Patient counseling is the most demanding part in the antenatal screening process, because it is not acceptable if medical personnel actively suggest to families “the right choice”. This is why information should be easily accessible, neutral and nondirectional. This does not simply mean the provision of brochures and information from the news media, but foremost the education of all the healthcare staff participating in antenatal care – from primary care centers to central hospitals. At all these levels counselors should actively help couples to make the right decisions for themselves (Clayton et al. 1995; Wertz and Gregg 2000; Pilnick and Dingwall 2001; Weil 2001). Nowadays the internet provides plenty of useful information for healthcare professionals, but it also provides information for parents, who in general are becoming more and more aware of issues related to their health. Considering the growing awareness of parents, the internet age has also become a new challenge for healthcare staff (Tarczy-Hornoch et al. 2000; Edwards 2001).

Preconception screening is recommended because it gives adequate time for genetic counseling and discussion of options. On the other hand, mothers do not usually think about fetal diseases before pregnancy (Clayton et al. 1996). If detection of these severe disorders is considered, we should offer genetic screening to all informed women, since screening only families who already have an affected child would result in detection of only a small fraction of affected fetuses. Our results demonstrate that genetic screening is acceptable and could be incorporated into prenatal clinics. Antenatal care provides a suitable gateway for such screening. Pregnant women are monitored on a regular basis; this implies a definitive advantage for genetic testing as the women are considering the health of their future offspring. The number of pregnant women at any one time is also relatively small, and therefore a genetic screening program would cover only a very small proportion of the total population.
According to experience in Tay-Sachs carrier screening, the screening programs have been most successful in relatively well-informed populations and where intrauterine diagnosis is available (Eng et al. 1997; Kronn et al. 1998; Mitchell et al. 1996; Zinberg et al. 2001). Education and providing people with information is essential, when improving understanding of genetic screening, and helping people to make their own decisions as to whether or not to participate in genetic screening (Eng et al. 1997; Welkenhuysen et al. 1996). There is also a need to improve the knowledge of healthcare staff, since they may not be familiar with genetic diseases (Schimpf and Domino 2001). Hence professionals need training in the special skills required to ensure a shared decision-making approach.

2. FRAGILE X SYNDROME AND THE FINNISH DISEASE HERITAGE

2.1 Fragile X syndrome

2.1.1 Clinical Features

Fragile X syndrome is the most common form of inherited cognitive impairment, and the second most common cause of mental retardation after Down’s syndrome, with an incidence of 1 in 4000 males or 2.4 in 10 000 males (Turner et al. 1996). Phenotypically, individuals can be divided into three groups: males with full mutation, females with full mutation and individuals with premutation.

Males inheriting the full mutation are phenotypically characteristic and they all have intellectual problems. Mental retardation in most prepubertal boys is moderate, the intelligence quotient (IQ) being 35 - 55, whereas it is moderate to severe in most of the adults, with IQ varying between 20 to 40 (de Vries et al. 1993; Curfs et al. 1991). Whether this apparent decline in IQ indicates a real loss of mental capacity or merely represents a slowing of cognitive development, stabilizing after puberty, is still unknown (Lachiewicz et al. 1987; Curfs et al. 1991; Fisch et al. 1991 and 1996; Hay et al. 1994). During the 1970s the clinical phenotype became delineated: long face, large protruding ears and macroorchidism in adult males (Escalante et al. 1971; Turner et al. 1975, 1978 and 1980; Cantu et al. 1976). In young fragile X boys the clinical phenotype is not so clear, but there are some physical characteristics, which include: an adverse response to a
touch on the skin, difficulty touching the lips with the tongue, soft skin over the
dorsum of the hand, and a hallucal crease (Lachiewicz et al. 2000).

Clinical findings in female Fragile X full mutation heterozygotes vary, but most of
them have mild to moderate retardation. Using DNA mutation analysis, 52 – 82%
of women heterozygous for the full mutation have been shown to have mental
impairment, the IQ being below 85 (Rousseau et al. 1991a and 1991b; Taylor et al.

The male and female carriers of FraX premutation are phenotypically normal
and they do not have intellectual problems; even rare female premutation
homozygotes appear to be normal (Mazzocco and Holden 1996). Currently, there is
no information on female full mutation homozygotes in the literature.

Behavioral problems and intellectual impairment dominate the clinical
presentation and intellectual impairment is not amenable to treatment. Dealing with
behavioral problems related to fragile X seems difficult, although behavioral therapy
and avoidance of strong stimuli may alleviate the symptoms. In some countries
intervention with medication is common, and a variety of drugs are in use,
examples being central nervous system stimulants such as dextroamphetamine,
tricyclics and clonidine (Hagerman 1996).

2.1.2 Molecular Biology

Fragile X syndrome originates from a lack of the protein product of the FMR1 gene
– FMRP (Pieretti et al. 1991). Recent studies have shown that FMRP is an RNA-
binding protein that shuttles between the nucleus and cytoplasm. This protein has
been implicated in protein translation as it is found to be associated with
polyribosomes and the rough endoplasmic reticulum (Tamanini et al. 1999, Jin and
Warren 2000). The precise function of FMRP is not yet resolved, but it is believed
that it is needed for neuron formation and synaptic connections, which are
important for development of the neuronal network, and thus for intelligence (De

The gene locus (FRAXA) of FMR1 is located on the distal long arm of the X
chromosome in region 2, band 7.3, Xq27.3 (Szabo et al. 1984). The cytosine-
guanine-guanine (CGG) repeat of the FMR1 gene is polymorphic in the normal
population, and varies between 6 and 55 units, with an average of 30 copies (Fu et al. 1991). There is an overlap in the range of 43 to 55 repeats between normal and premutated alleles. Only the finding of intra-familial instability in these intermediate alleles can identify them as premutation alleles (Eichler et al. 1994; Geva et al. 2000). Nolin et al. (1996) defined the premutation level as being 60-200 CGG repeats. This limit is still clinically widely used, although there have been rare reported cases where 58 or 59 CGG repeats have expanded to full mutation (Pembrey et al. 2001).

The stability of the premutation is dependent on the sex of the transmitting parent and on the size of the repeat. Transmission of the premutation through female meiosis may lead to enlargement of the repeat to above 200 units, full mutation, in the offspring. However, males with a premutation transmit the gene as a premutation to their phenotypically normal daughters (Sherman et al. 1985; Reiss et al. 1993).

There are also individuals simultaneously having both a full mutation and a premutation, often referred to as “size mosaics”. Individuals with intercellular variations in the methylation status of a full mutation are called “methylation mosaics”. In these two cases some level of FMRP production can be expected, leading to a less severe or even normal phenotype (Cohen et al. 1996).

2.1.3 Diagnosis

Historically, fragile X syndrome has been diagnosed cytogenetically. A secondary constriction on the distal long arm of the X chromosome was shown to be dependent on folate deficiency in the culture medium. It was localized to the interface between Xq27 and q28, and associated with mental retardation and macro-orchidism in males (Giraud et al. 1976; Harvey et al. 1977; Sutherland et al. 1977). This is not a suitable test for carrier detection, as approximately 50% of female carriers show no indication of fragile X in cytogenetic studies.

The diagnosis of fragile X syndrome is nowadays done by the PCR test, analyzing the CGG repeat lengths (Brown et al. 1993). If both alleles are of the same size (only one band) or when the amplification fails, Southern blot analysis is usually employed to rule out full mutation. The PCR test and selective Southern blotting are also used for prenatal diagnosis (Dobkin et al. 1991).
There is also FraX test which uses mouse monoclonal antibodies against the FMR protein. This way, diagnosis can be carried out from a blood smear, requiring only 1 or 2 drops of blood. This non-invasive test can be used for screening large groups of mentally retarded persons and neonates for fragile X syndrome (Willemsen et al. 1995, 1997).

2.2 The Finnish disease heritage

In Finland there are about 30 recessive diseases enriched in the relatively genetically isolated population – these diseases belong to the so-called Finnish disease heritage (Norio et al. 1973; Nevanlinna 1980; Norio 1981; Peltonen 1997; Peltonen et al. 1999). Diseases belonging to this group exist throughout the world, but are enriched in the Finnish population (Table 1).

A small population, a low population density and a high degree of national and regional isolation have generated a unique population structure in Finland (Norio et al. 1973; Nevanlinna 1980; Norio 1981). Loss and primary enrichment of rare genes were probably caused by the sampling (founder) effect or "bottle neck" and drift has been due to a numerically limited and slow immigration rate over the Gulf of Finland during the first centuries A.D. (Nevanlinna 1980).

The dual theory of inhabitation in Finland assumes an early migratory wave of eastern Uralic speakers some 4000 years ago, with a distinct effect on today’s Finnish gene pool. The majority of the genes of today’s Finnish population is thought to originate from later small founder populations which at the beginning of the first millennium immigrated from the south over the Gulf of Finland. This dual theory has recently been supported by analysis of Y chromosome haplotypes, which shows an exceptional decrease in the genetic diversity of the Finns when compared with other European populations (Sajantila et al. 1996).

Consanguinity is frequent between the parents of patients with rare autosomal recessive diseases. Closely consanguineous marriages are uncommon in Finland, and consanguinities indicate the unusual population structure of Finland rather than closely consanguineous marriages as the reason for homozygosity (Nevanlinna 1980).
<table>
<thead>
<tr>
<th>Disease (OMIM no.)</th>
<th>Defective protein</th>
<th>Loc us</th>
<th>Major mutation occurrence in Finland (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>APECED (240300)</td>
<td>Novel nuclear protein</td>
<td>82</td>
<td></td>
<td>(Niigamine et al. 1997)</td>
</tr>
<tr>
<td>Aspartylglucosaminuria (AGU, 208400)</td>
<td>Aspartylglucosaminidase</td>
<td>98</td>
<td></td>
<td>(Ikonen et al. 1991a)</td>
</tr>
<tr>
<td>Choroideremia (CHM, 303100)</td>
<td>Rab geranylgeranyl transferase</td>
<td>n.d.</td>
<td></td>
<td>(Sarkila et al. 1992)</td>
</tr>
<tr>
<td>Congenital chloride diarrhea (CCD, 214700)</td>
<td>Product of the gene down regulated in adenoma</td>
<td>100</td>
<td></td>
<td>(Höglund et al. 1996)</td>
</tr>
<tr>
<td>Congenital nephrosis (CNEF, 256300)</td>
<td>Nephrin</td>
<td>78</td>
<td></td>
<td>(Kestila et al. 1998)</td>
</tr>
<tr>
<td>Diastrophic dysplasia (DTD, 222600)</td>
<td>Sulfate transporter</td>
<td>90</td>
<td></td>
<td>(Hästbacka et al. 1994)</td>
</tr>
<tr>
<td>Familial amyloidosis, Finnish type (FAF, 105120)</td>
<td>Gelsoin</td>
<td>100</td>
<td></td>
<td>(Levy et al. 1990)</td>
</tr>
<tr>
<td>Gyrate atrophy of choroid and retina (HOGA, 258370)</td>
<td>Ornithine [gamma]-aminotransferase</td>
<td>85</td>
<td></td>
<td>(Mitchell et al. 1988)</td>
</tr>
<tr>
<td>Hypophosphatemic rickets (DHD, 258380)</td>
<td>Follicle stimulating hormone receptor</td>
<td>100</td>
<td></td>
<td>(Altmann et al. 1995)</td>
</tr>
<tr>
<td>Infantile neuronal ceroid lipofuscinosis (INCL, 256730)</td>
<td>Palmitoyl protein thioesterase</td>
<td>98</td>
<td></td>
<td>(Vesole et al. 1995)</td>
</tr>
<tr>
<td>Lysinuric protein intolerance (LPI, 222700)</td>
<td>L-Amino acid transporter</td>
<td>100</td>
<td></td>
<td>(Borsani et al. 1999)</td>
</tr>
<tr>
<td>Non-ketotic hyperglycinemia (NKH, 238300)</td>
<td>Glycine cleavage system; protein P</td>
<td>70</td>
<td></td>
<td>(Kure et al. 1992)</td>
</tr>
<tr>
<td>Progressive episcleritis with mental retardation (PEMR, 130143)</td>
<td>Novel transmembrane protein</td>
<td>n.d.</td>
<td></td>
<td>(Ranta and Lehesjoki 2000)</td>
</tr>
<tr>
<td>Progressive myoclonus epilepsy (PME, 254800)</td>
<td>Cystatin B</td>
<td>98</td>
<td></td>
<td>(Pennacchio et al. 1996)</td>
</tr>
<tr>
<td>Retinoschisis (RS, 312700)</td>
<td>XLR1</td>
<td>70</td>
<td></td>
<td>(Huoanier et al. 1999)</td>
</tr>
<tr>
<td>Sialic acid storage disease (SIASD, 268740)</td>
<td>Novel transporter</td>
<td>94</td>
<td></td>
<td>(Verheijen et al. 1999)</td>
</tr>
<tr>
<td>Finnish variant of late infantile neuronal ceroid lipofuscinosis (LNC, 256731)</td>
<td>Novel membrane protein</td>
<td>94</td>
<td></td>
<td>(Savukoski et al. 1998)</td>
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<tr>
<td>Cartilage-hair hypoplasia (CHH, 250250)</td>
<td>9q</td>
<td></td>
<td></td>
<td>(Sulisalo et al. 1993)</td>
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<tr>
<td>Cohen syndrome (COH1, 216550)</td>
<td>8q</td>
<td></td>
<td></td>
<td>(Tahvanainen et al. 1994)</td>
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<tr>
<td>Congenital lactase deficiency (CLD, 223000)</td>
<td>2q</td>
<td></td>
<td></td>
<td>(Järvelä et al. 1998)</td>
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<tr>
<td>Cornea plana congenita (CNA2, 217300)</td>
<td>12q</td>
<td></td>
<td></td>
<td>(Tahvanainen et al. 1995)</td>
</tr>
<tr>
<td>Fetal metabolic syndrome with iron accumulation (603356)</td>
<td>2q</td>
<td></td>
<td></td>
<td>(Visapää et al. 1998)</td>
</tr>
<tr>
<td>Hydrolithalas (236880)</td>
<td>11q</td>
<td></td>
<td></td>
<td>(Visapää et al. 1999)</td>
</tr>
<tr>
<td>Infantile onset spinocerebellar ataxia (IOSCA, 271245)</td>
<td>10q</td>
<td></td>
<td></td>
<td>(Nikoli et al. 1995)</td>
</tr>
<tr>
<td>Lethal congenital contracture-syndrome (LCCS, 253310)</td>
<td>9q</td>
<td></td>
<td></td>
<td>(Mäkelä-Bengs et al. 1998)</td>
</tr>
<tr>
<td>Meckel syndrome (MKS, 249000)</td>
<td>17q</td>
<td></td>
<td></td>
<td>(Paavola et al. 1995)</td>
</tr>
<tr>
<td>Muir-Torre syndrome (MUL, 253250)</td>
<td>17q</td>
<td></td>
<td></td>
<td>(Avela et al. 1997)</td>
</tr>
<tr>
<td>Muscle-eye-brain disease (MEB, 253260)</td>
<td>1q</td>
<td></td>
<td></td>
<td>(Cormand et al. 1999)</td>
</tr>
<tr>
<td>Presenile dementia with bone cysts (PLO-SL, 221770)</td>
<td>19q</td>
<td></td>
<td></td>
<td>(Peikarinen et al. 1998)</td>
</tr>
<tr>
<td>Selective intestinal malabsorption of vitamin B-12 (MAGA1, 261100)</td>
<td>10p</td>
<td></td>
<td></td>
<td>(Aminoff et al. 1995)</td>
</tr>
<tr>
<td>Tibial muscular dystrophy (TMD, 603034)</td>
<td>2q</td>
<td></td>
<td></td>
<td>(Haravuo et al. 1998)</td>
</tr>
<tr>
<td>Usher syndrome, type III (USH, 276902)</td>
<td>3q</td>
<td></td>
<td></td>
<td>(Sarkila et al. 1995)</td>
</tr>
</tbody>
</table>

* Online Mendelian Inheritance in Man, OMIM™. McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD), 2000.

**Major mutation occurrence in Finland; the percentage of affected alleles carrying the major mutation.
2.2.1 Congenital nephrosis of the Finnish type

The Finnish type of congenital nephrosis is one of the diseases belonging to the Finnish disease heritage. It is a rare autosomal recessive single gene disease, with a frequency of 1 in 8000 births in Finland (Huttunen 1976). In East Finland the birth prevalence is greater, being 1:2600 (Norio 1966). In non-Finnish populations the incidence is remarkably smaller, being approximately one in 40,000 (Crandall and Matsumoto 1991; Hogge et al. 1992). Clinical findings are massive proteinuria in utero, a large placenta and nephrosis from birth (Hallman et al. 1956 and 1967). The only treatment, which in most cases improves the prognosis remarkably, is kidney transplantation, but even after transplantation about 20% of the patients develop recurrent nephrosis, the cause of which is unknown (Holmberg et al. 1991 and 1995; Laine et al. 1993).

Congenital nephrosis was the first intrauterine condition in which an elevated amniotic fluid alpha-fetoprotein (AFAFP) level was described (Seppälä and Ruoslahti 1972). Even today CNF is screened conventionally by assay of maternal serum alpha-fetoprotein (MSAFP) at 15 - 16 weeks’ gestation. If the MSAFP level is elevated, then the amniocentesis is offered, to assay AFAFP (Heinonen et al. 1996).

Growing knowledge in genetics has lead to more precise antenatal diagnosis of CNF. The CNF gene has been localized to chromosome region 19q13.1 by linkage analysis and further studies revealed the NPHS1 (nephrin) gene (Kestilä et al. 1994; Männikkö et al. 1995). In Finland two mutations (Fin\textsubscript{major} and Fin\textsubscript{minor}) account for approximately 94% of mutated alleles: 78% of the Finnish NPHS1 chromosomes carry a 2-base-pair (bp) deletion in exon 2 (NPHS1 - Fin\textsubscript{major}) and 16% of the chromosomes carry a nonsense mutation in exon 26 (NPHS1 - Fin\textsubscript{minor}) (Kestilä et al. 1998). The nephrin gene belongs to the Ig superfamily and is expressed in the interpodocyte slit area (Ruotsalainen et al. 1999). So far a total of 36 mutations have been located within the gene worldwide (Lenkkeri et al. 1999). Lenkkeri et al. (1999) also reported that 20% (7/35) of non-Finnish cases have no detectable mutation along the nephrin gene sequence. These cases can be diagnosed prenatally by in utero kidney biopsy (Wapner et al. 2001).
2.2.2 Aspartylglucosaminuria – AGU

Aspartylglucosaminuria (AGU) is also an autosomal recessive disease, which belongs to the Finnish disease heritage. It results from the deficient activity of a lysosomal amidase, aspartylglucosaminidase (AGA). The estimated carrier frequency in Finland varies from 1 in 30 to 1 in 69 (Mononen et al. 1991, Hietala et al. 1993). The condition is manifested by excessive accumulation of uncleaved glycoasparagines, mainly aspartylglucosamine, in lysosomes, as well as elevated metabolite levels in the urine (Jenner and Pollitt 1967; Pollitt et al. 1968). An affected infant appears normal at birth and in early infancy. Developmental delay usually manifests before school age. The disease progresses slowly and leads to severe mental retardation and premature death in adulthood (Autio 1972; Aula et al. 1982).

The glycoasparaginase (GA) gene has been localized to chromosome region 4q32-q33 (Park et al. 1991; Morris et al. 1992). So far, a total of 25 mutations has been found in the world (Saarela et al. 2001). One major mutation (AGU\textsubscript{FinMajor}) has been identified in Finland which covers 98% of the affected alleles. This mutation consists of two nucleotide substitutions six nucleotides apart from each other (G482A and G488C), leading to two amino acid changes in the GA polypeptide (Arg161Gin and Cys163Ser, respectively) (Fisher and Aronson 1991; Ikonen et al. 1991a and 1991b; Mononen et al. 1991). The G488C mutation abolishes activity of the enzyme, while the G482A mutation is a neutral polymorphism associated with the G488C substitution. A two-basepair deletion in the GA gene (AGU\textsubscript{FinMinor}) can be recognized in 1.5% of Finnish AGU chromosomes (Isoniemi A et al. 1995). Currently there is no treatment for this disease, although the results of some studies on animals suggest that enzyme therapy may be helpful (Dunder et al. 2000).

2.2.3 Infantile neuronal ceroid lipofuscinosis – INCL

Infantile neuronal ceroid lipofuscinosis (INCL) is an autosomal recessive disease, and it causes the intralysosomal accumulation of lipopigments (Hagberg et al. 1968). The Finnish INCL mutation (INCL\textsubscript{Fin}) covers 98% of all patients in Finland and the carrier rate is 1 in 70 (Vesa et al. 1995; Syvänen et al. 1997). INCL leads to
severe neuronal destruction and mental retardation, death occurring in teenage years (Santavuori et al. 1973, 1974 and 1993; Uvebrant and Hagberg 1997).

The gene has been localized to chromosome region 1p32 (Järvelä et al. 1991; Hellsten et al. 1993). Mutations in a CLN1 gene encoding the hydrolytic enzyme palmitoyl protein thioesterase 1 (PPT1) are the underlying cause of INCL (Vesa et al. 1995). PPT1 is a lysosomal protein involved in removing fatty acids from palmitoylated proteins by deacylation (Camp et al. 1994). INCL is the most severe form of the neuronal ceroid lipofuscinoses (NCLs) and over 20 different mutations in the CLN1 gene have been identified in NCL patients worldwide (Hofmann et al. 1999). In the Finnish population, one mutation, a transversion of A to T at position 364 in the cDNA of CLN1, is INCL_Finn enriched (Vesa et al. 1995; Das et al. 1998). Mutation leads to the replacement of tryptophan with arginine in PPT protein, thus abolishing the enzyme activity. Due to the absence of PPT activity, cysteine-containing fatty acyl thioesters accumulate in the cells of INCL patients (Lu et al. 1996). It has been reported that the measurement of PPT activity permits the diagnosis of INCL patients as well as the detection of carriers of any mutation causing INCL (Cho et al. 1998; de Vries et al. 1999; van Diggelen et al. 1999). No specific treatment exists.
C. AIMS OF THE PRESENT STUDY

The aims of the present study were:

1. To investigate the applicability of prenatal diagnosis of fragile X syndrome in clinical practice and to investigate how the CGG repeat size of the premutation allele of the mother correlates with the risk of full mutation in the fetus.

2. To investigate pregnancy outcome among fragile X carrier mothers.

3. To investigate the utility and acceptance of genetic screening for congenital nephrosis of the Finnish type in antenatal care.

4. To investigate the applicability and acceptance of AGU and INCL screening in Finnish antenatal clinics.

5. To investigate the applicability of Fragile X, INCL and AGU gene tests integrated with traditional prenatal diagnoses based on fetal karyotyping.

In a nutshell, the hypothesis in this work was that genetic testing can be integrated into maternity care and could improve it.
D. MATERIALS AND METHODS

Individuals participating in the studies were pregnant women in the area of Kuopio University Hospital. The overall organization of the screening is presented schematically in Figure 1.

Figure 1. Screening methods used in maternity care in Kuopio since 1991.

Cascade screening (FraX) →
Down’s screening (AFP, hCG) →
Nuchal translucency ultrasonography →
CNF-screening →
INCL-screening →
AGU-screening →
Wide scope →

Year  91  95  96  97  98  99  00  01  02

1. Cascade screening refers to an index case-based search for fragile X families, which has been going on in the clinical genetics unit of Kuopio University Hospital (KUH).
2. Down’s screening refer to biochemical Down’s syndrome screening, which has been in use in antenatal care.
3. Nuchal translucency ultrasonography refers to ultrasonographic screening for Down’s syndrome in KUH; CNF screening refers to the congenital nephrosis gene test, used in antenatal screening.
5. Wide scope refers to a study, where fragile X-, INCL- and AGU- gene tests were integrated with prenatal diagnosis and fetal karyotyping.

For each study, a specified subgroup was included as follows:

Aim I: In 89 pregnancies with an increased maternal risk of fragile X syndrome, chorion villus or amniotic fluid samples were tested for fragile X gene status. These women were from three different sources:

1. Women who had a known fragile X-affected relative were offered a gene test. This is called cascade screening, referring to an index case-based
search for fragile X families, which has been going on in the clinical genetics unit of Kuopio University Hospital (KUH).

2. **Antenatal genetic screening was offered to all pregnant mothers in maternity care units.**

3. **Fragile X gene testing was also offered to women undergoing prenatal diagnosis for some other reason.**

   All women with an increased number of CGG repeats were informed of the possibility to have prenatal diagnosis of the fragile X gene status of the fetus.

**Aim II:** When studying pregnancy outcome in fragile X carrier women, we collected information retrospectively in regard to 63 pregnancies of fragile X carrier women (eight women had a full mutation and 55 had a premutation) among those seeking genetic counseling and prenatal diagnosis at the University Hospital of Kuopio. The reference group was derived from 8596 control women who gave birth at the University Hospital of Kuopio from January 1995 to December 1998.

**Aim III:** Gene test-based CNF screening was offered to a total of 1303 pregnant women at same time as assessment of first trimester nuchal fold translucency, from January 1999 to October 1999. Genetic testing was important, because at this time there was a pause in biochemical AFP-based congenital nephrosis screening.

**Aim IV:** Gene tests for AGU and INCL were offered to pregnant mothers to diagnose possible carriership, in maternity care clinics at health centers. The AGU test was offered to 3335 pregnant women and the INCL test to 2626 women, from January 1995 to December 1996.

**Aim V:** In the wide scope prenatal diagnosis study there was a total of 260 women who underwent chorion villus sampling (CVS) or amniocentesis at Kuopio
University Hospital during the study period, from January 1997 to December 1998. They were also offered gene tests to diagnose possible carriership of FraX, AGU and INCL. Of these, 239 were potential participants in genetic testing, since they had not undergone carrier screening previously. Three mothers who had more than one pregnancy during the study period were tested only once. Two of the tested mothers had twin pregnancies.

All five studies presented here were approved by the Research Ethics Committee of Kuopio University Hospital. The healthcare providers, mostly midwives, trained by geneticists, gave genetic counselling to all pregnant women in regional maternity centers. Before offering the gene test, all women received a brochure describing the clinical picture of CNF, fragile X syndrome, or AGU and INCL. When genetic testing was offered, the voluntary nature of participation in screening was emphasized. All subjects gave verbal or written consent before being enrolled in the studies, the exception being the pregnancy outcome study, which was carried out retrospectively on the basis of patient files. Information on the genetic screening program was also disseminated through news media and by advisory visits to the maternity care units in the area.

In all studies, where new patient-specific genetic data were obtained, the files containing this data were kept separate from patient files used for clinical work. The aim of this was to maximize information security. Only the subject concerned decided whether or not this information was to be used for other purposes, such as documents for insurance companies, other doctors, relatives, the government, etc. Otherwise, organizations/people did not have access to the individual’s genetic information.

In all four studies where new genetic information was gathered, the gene tests were offered free of charge by the hospital. All blood samples were taken in our hospital or in primary care centers to be analyzed for AGU and INCL carrier status at the Department of Clinical Chemistry of Helsinki University Hospital, and for fragile X or CNF carrier status at Kuopio University Hospital Chromosome and DNA laboratory. The minisequence test described by Syvänen et al. was applied for AGU and INCL tests (Syvänen et al. 1997, 1992). Two PCR-based tests applying to CNF are described in detail in the original paper (III). If the mother was shown to be a carrier, the appropriate gene test was also offered to the male partner. If both parents
were found to be carriers, we offered the testing of fetal genotype from cultured trophoblastic cells or amniocytes. The FraX mutation was tested for as described by Brown et al. (1993), and PCR testing and selective southern blotting (Dobkin et al. 1991) were also used for prenatal diagnosis.
E. RESULTS

1. PRENATAL DIAGNOSIS OF FRAGILE X SYNDROME AND THE RISK OF EXPANSION OF PREMUTATION

In the first study we investigated the pregnancies of 89 fragile X carriers. Three of the pregnancies were aborted. All aborted pregnancies showed that the fetus had full mutation. In our hospital we held to the policy that if amniotic fluid or chorionic villus biopsies were taken for assessment of fragile X status, we also investigated the chromosomal status of the fetus, although we did not use cytogenetic diagnosis for fragile X syndrome. Fetal karyotyping revealed two abnormalities, genotypically: 47XXY and 45X.

In three cases, where a mother’s CGG repeat in the FMR1 gene was below premutation level, at 40 - 60 (N = 32), there was expansion to premutation in the fetus (53 → 61, 56 → 76, 57 → 62). There was no expansions to full mutations in this group. When the mother’s premutation was between 60 and 80 repeats (N = 21), the risk of fetal full mutation was 4.8 %. If the fetus inherited the premutated allele, the risk of full mutation was 14 %. If the mother’s premutation size was 80 – 100 repeats (N = 13) the overall risk of fetal full mutation was 61.5 %, and if the fetus inherited the mutated allele, the risk of full mutation was 89 %. In the mothers with full mutation (N = 22), 36 % of the fetuses inherited a normal FMR1 gene; the rest inherited the mutated allele. There was no relationship between full mutation and fetal sex.

2. PREGNANCY OUTCOME IN FRAGILE X CARRIERS

There were 63 pregnancies of fragile X carrier women (8 had full mutation and 55 had a premutation). There were no differences in maternal risk factors in comparison with the reference group. The study revealed no statistically significant differences when the occurrence of placental abruptio, pre-eclampsia, cesarean section, forceps/vacuum extraction, meconium-stained amniotic fluid, admission to a neonatal unit, intrauterine fetal death, prematurity, low birth weight, fetal distress and small-for-gestational age fetuses were investigated. Late pregnancy bleeding was an exception, and a statistical significance was revealed, although only two
cases in the carrier group were found. The adjusted risk of late pregnancy bleeding increased from 1.0 (reference group) to 5.84 (95% CI 1.39-24.6). Infant health was similar in the two groups. An interesting, but statistically non-significant finding was that the proportion of female infants in the study group was higher than the control group.

3. ANTENATAL GENETIC SCREENING FOR CONGENITAL NEPHROSIS

A total of 1183 pregnant mothers were tested for CNF carrier status. The test was incorporated in the ultrasonographic antenatal screening for fetal trisomy in the first trimester. The acceptance rate was high, being 91% of all 1303 pregnancies. Thirty-eight women were found to be carriers of the disease. Of these, 34 (89.5%) had Fin\textsubscript{major} and four (10.5%) had Fin\textsubscript{minor} mutations. All partners of the carrier women were tested and two of them were found to be carriers of the Fin\textsubscript{major} mutation. The two couples requested further testing, and invasive prenatal diagnosis was carried out. One fetus was a CNF carrier and the other was affected. The affected pregnancy was legally terminated. The carrier frequency appeared to be 1 in 31, which would theoretically mean an incidence of 1:3844 in East Finland.

4. ANTENATAL GENE TESTS IN LOW-RISK PREGNANCIES: MOLECULAR SCREENING FOR ASPARTYLGLUCOSAMINURIA (AGU) AND INFANTILE NEURONAL CEROID LIPOFUSCINOSIS (INCL) IN FINLAND

During the study period of two years, we found 47 (1.4%) female AGU carriers and 14 (0.5%) INCL carriers. These figures represent a carrier frequencies of 1:62 for AGU and 1:163 for INCL. The acceptance rate was good, the overall uptake of gene tests being 87%. One of the tested male partners was an AGU carrier and prenatal diagnosis revealed a carrier fetus. None of the tested male partners was an INCL carrier.
5. WIDE SCOPE PRENATAL DIAGNOSIS AT KUOPIO UNIVERSITY HOSPITAL 1997 - 1998; INTEGRATION OF GENE TESTS AND FETAL KARYOTYPING

We offered comprehensive prenatal diagnosis including fetal karyotyping and gene tests for AGU, INCL and FraX to all women eligible for amniocentesis or chorion villus biopsy because the estimated risk of abnormal pregnancy was considered to be higher than usual. Maternal age, a positive biochemical or genetic test screening result and a previous history of abnormal pregnancy were taken into account when estimating maternal risk. The chromosome findings revealed no abnormalities in 241 fetuses (94.1 %); in four cases there was no result (culture failure), and in 15 (5.9 %) cases they revealed chromosomal abnormalities: four with trisomy 21, two with trisomy 18, three with 46XX/XY translocation, one with 47XYY, one with 47XXY, one with 45X, one with 45XX translocation, one with 69XXX and one with 47XY translocation.

Altogether, 220 of 239 eligible women elected to undergo the gene tests offered. Thus the acceptance rate was high: 92.1 %. We found seven AGU carrier women, and in one couple, where both parents were carriers, the fetus was tested and appeared to be unaffected. Tests for INCL revealed 2 carrier women, but their partners appeared to be unaffected. Fragile X screening revealed only one carrier woman, with a small premutation of 62 repeats. Prenatal diagnosis of the fetus revealed that it was unaffected.
F. DISCUSSION

We investigated whether genetic testing could provide us with better ways to carry out prenatal diagnosis, and thus improve the results of prenatal screening, previously based on biochemical markers and maternal age. Genetic testing is now coming into clinical use in many laboratories and hospitals throughout the world and at the very least it should be considered when a woman is seeking prenatal diagnosis and undergoing invasive testing. The clinical significance of the disorders and the availability of high quality genetic screening tests were essential aspects when these studies were designed, and therefore FraX, AGU, INCL and CNF were selected for this pilot study. Fragile X syndrome is the second most common cause of mental retardation, whereas AGU, INCL and CNF belong to the so called “Finnish disease heritage”, meaning that all these diseases have an impact on public health and are serious enough to be screened for. The pregnancy outcome of FraX mothers was investigated because the results of previous studies have suggested that premutation carriers have gynecological disorders such as early menopause more often than the general population, thus possibly indicating to problems concerning pregnancy (Schwartz et al. 1994; Partington et al. 1996). Antenatal genetic screening in maternity care is taking it’s first steps and no permanent public screening programs have been established in Finland. The major factor that inhibits gene screening is that we do not have any curative treatment for hereditary diseases. Genetic screening will be seen in a new light when treatment develops. With advanced gene technology we are at the beginning of a new era; the day is not so far off when it will be possible to cure at least some genetic diseases or offer gene therapy in utero, instead of abortion (Zhong and Wisniewski 2001).

Genetic screening is commonly complicated by the fact that the disease gene may have many different mutations leading to similar clinical pictures. For instance, in cystic fibrosis over 900 mutations in the cystic fibrosis transmembrane conductance regulator gene have been identified (Roque et al. 2001). This represents a great challenge to the development of carrier screening programs. However, common mutations are often enriched and occur at greater frequency in different populations. This also holds true with regard to the Finnish disease heritage, where only two or three main mutations are found in most diseases and they account for 98 – 99 % of the disease phenotypes. For instance, in AGU one
major mutation is responsible for 98% of the mutant alleles in the entire population in Finland.

1. THE EXPANSION RISK OF FMR1 PREMUTATION INCREASES WITH MATERNAL PREMUTATION SIZE

In the study, where prenatal diagnosis of fragile X syndrome was investigated, maternal premutation size was positively correlated with the number of CGG repeats in the offspring. The high proportion of female infants in the study group might reflect the high spontaneous abortion rate of male fetuses with full mutation. It also appeared that if the mother had a large premutation (> 80 repeats) or a full mutation, the probability of the fetus inheriting the normal allele was somewhat lower than among those mothers having a normal number of repeats or a low-range premutation, since only 31% of women with a large premutation and 36% of women with a full mutation transmitted the normal allele to their offspring. However, this may be just a coincidence, occurring as a result of the small number of such pregnancies, and further prospective studies are needed to clarify this issue in the future. The results of this study revealed no risk of having a full mutation fetus with a maternal CGG repeat number below 60. Nevertheless, there are cases in the literature where CGG repeats below 60 have expanded to a full mutation. On the other hand, the risk of complications related to invasive prenatal testing is still considered higher than the risk of a full mutation, and therefore a CGG repeat number of 60 is used as a limit as regards carrying out invasive prenatal diagnosis in clinical work (Pemberay et al. 2001). There remain problems, such as a full mutation in a female fetus, where we can not accurately predict the mental state of the offspring.

2. FRAGILE X CARRIERS HAVE GOOD PREGNANCY OUTCOMES

The pregnancy outcome in fragile X carriers revealed no statistically significant differences when various pregnancy and delivery complications in the study group and the reference group were investigated, with the exception of late pregnancy bleeding. We found an increased risk of late pregnancy bleeding in the carrier group and further investigations will show whether this finding is real or merely
coincidence. The overall health of the infants was similar in both groups. Women with fragile X carrier status should be screened for fetal expansion of the premutation, but the results of this study revealed no need to initiate further special fetal monitoring because of maternal fragile X carrier status. It has been suggested that fragile X carrier status may be associated with gynecological complications, especially early menopause (Schwarz et al. 1994; Partington et al. 1996), and mental retardation of full mutation mothers could affect their behaviour during pregnancy. The low number of fragile X carrier pregnancies (n = 63) in this study makes it difficult to draw any firm conclusions about the maternity care of fragile X mothers. Some fragile X full mutation mothers with mild to moderate mental retardation are probably in need of extra care and support in primary maternity care units during pregnancy.

3. ANTENATAL GENETIC SCREENING FOR CONGENITAL NEPHROSIS HAS BENEFITS OVER AFP SCREENING

Congenital nephrosis of the Finnish type was selected as a pilot disorder to audit screening, since antenatal screening for this disease is standard practice in Eastern Finland (Rynänen et al. 1983; Heinonen et al. 1996). Acceptance of the CNF carrier gene test in maternity care units was good, the uptake being 91 %. The carrier rate for CNF was found to be 1:31, meaning that there is one couple in 961 where both parents are carriers. Since one in four of their children is affected, approximately one in 3844 children born has CNF. These figures are in line with those of previous studies (Huttunen 1976, Norio 1966). When comparing the CNF gene test with AFP-based biochemical screening, the former is preferable, because the latter method suffers from occasional false-positive results (Heinonen 1996).

4. GENE TESTS ARE APPROPRIATE FOR SCREENING OF ASPARTYLGLUCOSAMINURIA AND INFANTILE NEURONAL CEROID LIPOFUSCINOSIS IN ANTENATAL CARE

In regard to antenatal gene tests for AGU and INCL, the acceptance rate was found to be good, the overall uptake being 87 %. The carrier rate for AGU was found to be 1:62, meaning that approximately one in 15,376 children is born with the disease,
which figure in turn is in line with those of previous studies in Finland (Hietala et al. 1993). The carrier rate for INCL was found to be 1:163, which is somewhat lower than figures found in previous studies (Vesa et al. 1995). On the basis of experiences in Kuopio, it seems feasible to incorporate the minisequencing test in antenatal screening protocols in maternity care. This way, the incremental costs of testing and counseling are moderate.

5. WIDE SCOPE PRENATAL DIAGNOSIS PROVIDES FUTURE PARENTS WITH FURTHER ASSURANCE

With genetic screening offered to 260 women undergoing invasive prenatal diagnosis, a total of 10 new carriers of serious genetic diseases were found: two INCL carriers, seven AGU carriers and one with FMR1 premutation. Almost all women accepted the gene tests and they had a favorable attitude towards genetic testing. All the women who participated in the screening process were seeking invasive prenatal diagnosis to ensure the birth of a healthy child. When a pregnancy is potentially exposed to miscarriage by invasive testing, and fetal chromosomes only are examined, the investigation is far from complete. In the case of a genetic defect, families find it difficult to understand the birth of a seriously ill child, when fetal karyotyping appeared to be normal. If we sample fetal material and risk the fetus, it is logical to carry out a comprehensive investigation on fetal cells in order to investigate as many severe fetal diseases as possible (Brambati et al. 1998). When a mother is seeking invasive prenatal diagnosis, she is already prepared, at least to some extent, for “bad news”. Genetic screening in such cases does not carry the same ethical problems as when future parents unexpectedly face a positive screening test result. The high acceptance rate of gene tests in this study reflects the opinion of a selected population and is not suitable as a reference when a population-based screening program is considered.

6. GENETIC SCREENING AS A PART OF REPRODUCTIVE CARE

Earlier, we had no clue as to which specific test should be carried out for most genetic conditions. Now the spectrum of diagnostic possibilities in prenatal diagnosis is changing - hand in hand with the giant leaps in molecular biology and the human genome project (Semsarian and Seidman 2001, Quackenbush 2001). Over the past
years, there have been a number of publications on the cloning of genes for Finnish inherited diseases (Table 1). Theoretically, prenatal diagnosis is possible as regards these diseases, as with so many other Mendelian disorders where the mutation can be identified. Currently the options open to women with a genetic disease are: 1) oocyte donation, 2) preimplantation diagnosis, and 3) invasive prenatal testing. There may also be many asymptomatic women who feel unable to risk having children because of these uncertainties involved. The major advantage of antenatal genetic testing is its ability to identify the carriers of different diseases in a timely fashion so that they can have appropriate genetic counselling and prenatal testing.

Screening of fetal cells in maternal blood may be of value in the future, although nowadays it is in its early stages. Fetal cells can be identified in the maternal circulation and techniques such as fluorescence or magnetic in situ hybridization can be used to identify aneuploidies, including Down's syndrome and trisomy 18 (Wald et al. 1997, Geifman-Holtzman 2000). Sekizawa et al. (1999) have introduced a method where diagnosis of fetal gender and RhD genotype have been successfully determined by combining fluorescence in situ hybridization and PCR. The technique they describe holds the promise of noninvasive diagnosis of recessively inherited single-gene disorders. Discoveries of gene defects in the Finnish disease heritage will enable us to detect carriers of many severe hereditary diseases. It can be anticipated that understanding of the transmission genetics and segregation of DNA mutants will be revolutionized by new methods based on the DNA array techniques currently being developed and that large-scale prenatal diagnosis and antenatal screening will become routine thereafter (Ramsay 1998; Pastinen et al. 2000).

Attitudes towards genetic testing have become favorable in Finland. However, a commonly expressed reason given against testing was that test results might lead to discrimination in employment or insurance (Hietala et al. 1995). This kind of concern can be alleviated with specific medical records and solutions provided by novel information technology. For example, in some ultra-religious Jewish communities, where arranged marriages are still common, there is a confidential database and even the individuals concerned are not informed of gene test results. When a potential match is proposed, the rabbi's office is contacted and if the potential couple is at risk, the pair is considered incompatible (Zinberg et al. 2001). When attitudes towards population-based screening for cystic fibrosis were investigated in Australia, it was found that women, younger people, people with higher education, people without
children, and people planning to have children were more likely to undergo testing (Honnor et al. 2000). Experiences in Ashkenazi Jewish community screening indicates that only through genetic counseling and education can screening in the general population gain wide acceptance and provide maximum benefit (Zinberg et al. 2001). On the other hand, genetic information might be useful for insurance companies, banks, and certain authorities and employers. Because of this, in our hospital genetic information gained in these tests is kept separate from patient files, and may only be accessed by research staff and the individual her/himself. If genetic testing is broadened, there should be legislation to protect the rights of the individual.

The problem of appropriate counselling in expectant mothers before and after decision-making is of utmost importance. It is also essential to discuss the "sequence of decisions" before the offer of a screening test, since a positive result will lead to decisions as to whether or not to undergo more detailed diagnostic testing and whether or not to continue the pregnancy. Furthermore, the recommendations depend on the particular mutation and hence it is important for each pregnant subject that specialist advice be sought in counselling. It has been reported that the percentage of women accepting prenatal diagnosis is not related to the percentage of women who would terminate their pregnancy on the basis of prenatal diagnosis (Eurogapppp project 1999-2000). In France, only 80% appeared to have received appropriate information, although 97% of all women signed a consent form before maternal serum screening (Eurogapppp project 1999-2000). The same problems as with trisomy screening tests are also evident with gene test-based screening. Important questions to answer are who should be offered testing and in what setting, how should the lack of public understanding of genetics be dealt with, how should the insufficient number of trained health care providers be increased, how can individual autonomy be safeguarded, and what kind of procedures should be undertaken to ensure the quality of genetic counselling (Eurogapppp project 1999-2000).

A consensus has been reached on the fact that informed choice should be the basis of every genetic screening program. Because the majority of the population is as yet relatively uninformed, counseling is necessary to safeguard individual autonomy and choices. Health professionals’ awareness and knowledge of genetics need to be improved. Collaboration with patient organizations is also needed to ensure that information surrounding the offer of a screening test reflects the perceived
impact of the condition from the perspective of those affected (Eurogapppp project 1999-2000). The Ministry of Social Affairs and Health in Finland has also given guidelines to arrange population-based screening programs in Finland. These guidelines also underline the fact that, screening should be equally available to all people in the target population, which is best accomplished through the primary health care system. The ministry also sees that there are insufficient scientific findings to support the screening of multifactorial genetic diseases (Geeniseulontatyöryhmän muistio 1998).
G. CONCLUSIONS

The results of the present study strengthen the view that fragile X carrier mothers should be offered invasive prenatal diagnosis only when the mother’s CGG repeat number is over 59. Otherwise a fragile X syndrome carrier should be advised, that there are no other indications for special fetal monitoring, and the pregnancy outcome is generally favorable.

Studies on the antenatal screening of CNF, AGU and INCL showed that genetic testing is suitable and well accepted in antenatal clinics in Eastern Finland. This implies that when a screening program like this exists and is well organized, the participation rate is high and it satisfies a genuine need. To detect difficult recessive autosomal disorders, genetic carrier screening is preferable to cascade screening, because a carrier status as regards recessive inherited disorders is very common, but an affected child is born only when both parents by chance carry a recessive defect of the same gene. In other words, the effectiveness of cascade screening is limited if only the relatives of affected individuals are screened. Preconceptual screening would theoretically be preferable. However, in practice concern about a child’s health arises more often in early pregnancy, when mothers face the need of fetal diagnosis, and therefore antenatal clinics are a suitable gateway for such a screening process.

The study concerning genetic screening incorporated with fetal karyotyping indicated that much can be done to improve invasive prenatal diagnosis. Genetic testing should be incorporated with prenatal diagnosis to provide the mother with as accurate a picture as possible. The birth of a seriously ill child with a genetic disorder is always very hard to understand, when invasive prenatal diagnosis showed a normal karyotype.

In conclusion, genetic testing is now a well established part of medical care which may benefit a number of different groups of individuals. In maternity care in particular it should be more widely available. The essential concern in all kinds of screening is the family, not the community or the medical profession and it is unethical to pressure the people to participate in screening. Further studies are needed for better understanding of the effects and consequences of rapidly developing gene tests in clinical reproductive medicine.
H. REFERENCES


76. Hästbacka J, de la Chapelle A, Mahtani MM, Clines G, Reeve-Daly MP, Daly M, Hamilton BA, Kusumi K, Trivedi B, Weaver A et al. The diastrophic


I. ORIGINAL PUBLICATIONS I TO V