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Original article

Congenital nephrotic syndrome of the Finnish type: linkage to the locus in a non-Finnish population

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Abstract. Congenital nephrotic syndrome of the Finnish type (CNF) is inherited as an autosomal recessive trait. The biochemical basis of the disease is unknown, although a lesion in the glomerular basement membrane is strongly suggested. Recently, the CNF locus was assigned to chromosome 19q12–q13.1 on the basis of linkage analysis in Finnish families. The high incidence of the disease in Finland, as well as the demonstration of linkage disequilibrium in the Finnish study, strongly suggests a founder effect based on a common ancient mutation in this population. We confirm linkage of the CNF locus to the same chromosomal region in seven non-Finnish CNF families without evidence of linkage disequilibrium. Our results show that the same gene seems to be affected in both Finnish and non-Finnish CNF populations. However, in the latter the mutation-carrying chromosomes descend from different ancestors without evidence of a founder effect.

Key words: Congenital nephrotic syndrome – Chromosomal localization – Linkage – Disequilibrium – Homozygosity mapping

Introduction

Congenital nephrotic syndrome of the Finnish type (CNF) is the most common of all types of congenital nephrotic syndromes. It is inherited as an autosomal recessive trait. Although CNF occurs most often in Finland, with an estimated incidence of 1.2 in 10,000 pregnancies [1], typical cases of CNF have been reported from all over the world. Most infants with CNF are born prematurely and present with characteristic clinical features: large placenta weighing more than 25% of the baby's birth weight, massive proteinuria starting in utero, and severe steroid-resistant nephrotic syndrome. The most characteristic histological findings are dilated proximal tubules, mesangial hypercellularity, and glomerular fibrosis and sclerosis [2]. The disease may be diagnosed prenatally by measurements of alpha-fetoprotein (AFP) concentrations in amniotic fluid at 16–20 weeks of pregnancy [3] and in maternal serum [4]. However, false-negative amniotic AFP levels have been reported, and elevated maternal serum AFP levels are less reliable.

The pathogenesis of the CNF is still unknown, but it has been postulated that the primary defect leading to the nephrotic syndrome could be a structural and functional defect in the glomerular basement membranes. However, Kestilä et al. [5] excluded some candidate genes coding for basement membrane-specific components as mutated loci in CNF, like the α1–α5 chains of type IV collagen, different laminin chains, and the heparan sulfate proteoglycan core protein, as well as the Pax-2 gene [6], involved in the development of congenital nephrotic syndrome in transgenic mice [7]. Recently the same group assigned the locus for CNF to human chromosome 19q12–q13.1 on the basis of linkage analysis in 17 Finnish families [8]. This raised the question of whether the same gene could be involved in non-Finnish CNF populations. We confirm linkage to this region by examination of seven additional CNF families of European and Northern African origin.

Patients and methods

Patients. Blood samples were obtained from seven simplex families with CNF, following informed consent of all individuals or of their parents (Fig. 1). The families originated from France (families 2 and 3), Germany (4), Turkey (5 and 6), and Northern Africa (1 and 7). The diagnosis was based on the following criteria: severe proteinuria at birth with nephrotic syndrome appearing during the first weeks of life, large placenta, and histological pattern on renal biopsy. Renal tissue (5×biopsy specimen and 2×autopsy specimen) was studied in at least one patient per family. Diffuse mesangial hypercellularity with increase in mesangial matrix and focal dilatation of proximal tubules were observed in all patients. More or less severe degenerative changes consisting of focal and segmental and/or focal and global glomerulosclerosis, tubular atrophy, and interstitial fibrosis were always present.

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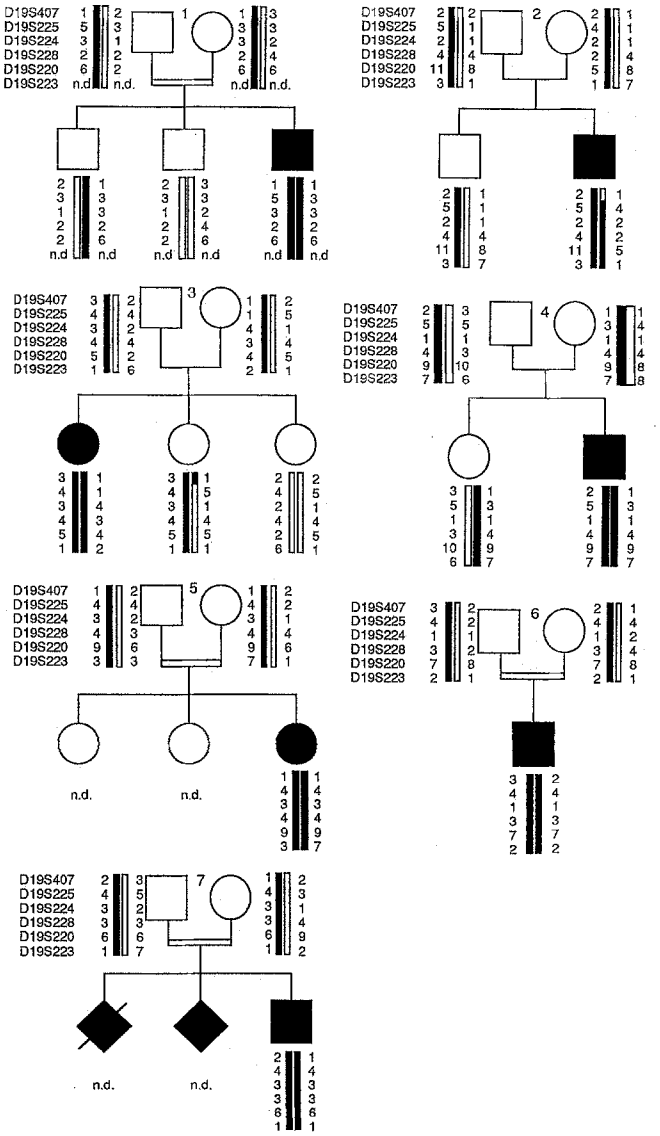

Fig. 1. Pedigrees of seven families with congenital nephrotic syndrome of the Finnish type (CNF) including the haplotypes observed with the six markers D19S407, D19S225, D19S224, D19S228, D19S220, and D19S223. Families 1 and 7 are of Northern African, families 2 and 3 of French, family 4 of German, and families 5 and 6 of Turkish origin. n.d., Not determined

A total of 29 meioses were assessed, 7 of which were affected individuals. Parental consanguinity was found in the Turkish and Northern African families.

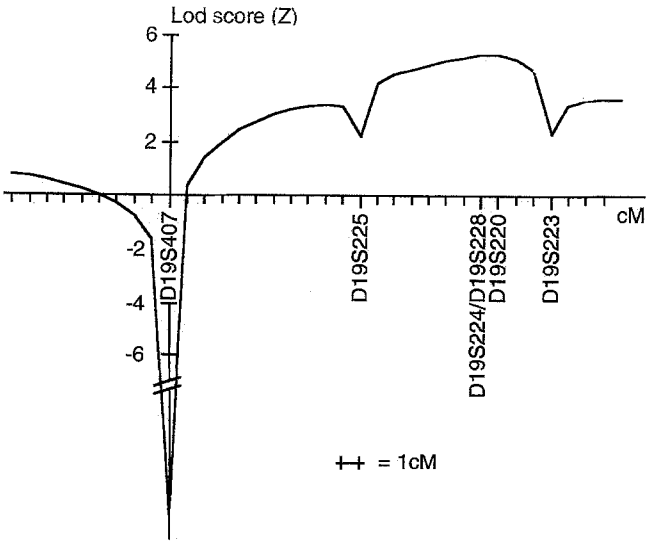

Fig. 2. Multipoint linkage analysis with six markers in seven CNF families. Although recombination events were not observed between markers D19S220 and D19S228, the recombination fraction was set to 0.001

Linkage analysis. Genomic DNA was extracted from leukocytes according to standard laboratory protocol. Polymorphic microsatellite markers were analyzed by polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis. PCR was performed in a volume of 25 µl using 50 ng of genomic DNA, 50–100 ng of the specific primers, and 0.2 U of *Taq* polymerase (ATGC) in 1×*Taq* buffer (ATGC). Genotyping was performed either with fluorescence-based techniques using an automated 373A DNA sequencer with Genescan 672 and Genotyper Software (Perkin Elmer) [9] or by radiolabelling using primers originating from Généthon [10].

Genetic linkage analyses was carried out using the FASTLINK package, version 2.2 [11, 12]. Allele frequencies were calculated by adding the allele frequencies obtained in our population to those found in the genome data base. Penetrance was assumed to be complete. Multipoint linkage analysis was carried out by applying the strategy of homozygosity mapping using the program MAPMAKER/HOMOZ [13]. The program LODVIEW [14] was used for the evaluation of lod score results.

Results

Six microsatellite markers located in the CNF region on chromosome 19q12–q13.1 were tested for linkage in seven families fulfilling the diagnostic criteria for CNF. The order of the markers tested was based on previously published

Table 1. Two-point lod scores (Z) between congenital nephrotic syndrome of the Finnish type (CNF) locus and chromosome 19 markers of all CNF families

	Recombination fractions θ								
	0.0	0.01	0.05	0.08	0.1	0.15	0.2	Zmax	θmax
Locus									
D19S407	−∞	−2.55	−0.78	−0.40	0.25	0.06	0.01	0.03	0.712
D19S225	−1.56	−0.14	0.31	0.34	0.33	0.27	0.19	0.34	0.081
D19S224	2.93	2.83	2.43	2.14	1.96	1.53	1.16	2.93	0.0
D19S228	2.46	2.37	2.04	1.81	1.66	1.30	0.99	2.46	0.0
D19S220	3.79	3.68	3.22	2.89	2.68	2.17	1.71	3.79	0.0
D19S223	−1.42	0.04	0.44	0.46	0.45	0.39	0.31	0.46	0.075

data [8, 10]: centromere – D19S407 – D19S225 – D19S224 – [D19S228 – D19S220] – D19S223 – telomere (the parentheses indicate that the precise order is unknown). The highest lod scores were obtained with markers D19S224, D19S228, and D19S220, with a maximal lod score of 3.79 at θmax = 0.0 to D19S220 (Table 1). There was no evidence of genetic heterogeneity. Haplotype analysis suggested that the gene is located between markers D19S225 and D19S223. The most likely position in this interval was calculated using multipoint analysis and was found to be at θ = 0.0 for D19S228/D19S220 (multipoint Zmax = 5.40) (Fig. 2). None of the tested markers showed a significant allelic association with CNF chromosomes.

Discussion

Since the pathogenesis of CNF remains unknown, the first step towards the identification of the gene is the positional cloning approach using linkage analysis. Recently Kestilä et al. [8] assigned the CNF locus to the long arm of human chromososme 19, by testing 17 Finnish CNF families. The frequency of CNF is unusually high in Finland, compared with other countries. Since it is assumed that the Finnish population has expanded from a small number of founder individuals 2,000 years ago to 5 million today [15], about 30 diseases, including CNF, are known today to belong to a group of mostly recessive disorders that occur in greatly increased frequencies in Finland [16]. Other disorders with evidence of a founder effect, such as Friedreich ataxia among Louisiana Acadians [17] and myotonic dystrophy among French Canadians [18], have been reported. It has been demonstrated that linkage disequilibrium is a powerful tool for refining a genetic map in isolated founder populations. In these cases the ancestral mutation has segregated from a common chromosome so that the affected chromosomes show a distinctive haplotype in the immediate vicinity of the gene. The Finnish group demonstrated linkage disequilibrium with two markers, which significantly increased the respective lod scores and also strongly suggested a founder effect. These results are in perfect concordance with the high incidence of the disease in this country.

This raised the question of whether the same gene would be involved in CNF families of other than Finnish origin, thus suggesting them to be part of this genetically distinct population. We therefore performed linkage analysis in seven European and Northern African CNF families. Linkage analysis in recessive traits usually requires the availability of families with multiple affected individuals. However, it is known that recessive traits occur more often in consanguinous families, due to homozygosity by descent. In these cases an affected individual is usually homozygous for the mutated allele, since both the maternal and the paternal alleles are supposed to be inherited from a common ancestor. Thus, the detection of these regions of homozygosity by descent (homozygosity mapping) permits the localization of a disease locus even in small families with only one affected inbred child [19]. Using this method – supported by an accurate computer program [13] – we were able to obtain a significant lod score with a small population of only seven simplex families, since four of them were inbred pedigrees.

Our results confirm linkage of the CNF locus to the chromosomal region 19q12–q13.1, without evidence of genetic heterogeneity. The localization of the CNF gene represents the first step towards the identification of the gene itself. The characterization of the gene will probably provide new insights into the pathogenesis of the disorder. Unfortunately, none of the genes identified to date which code for glomerular basement membrane components are localized in this region. Consequently the approach for the identification of the gene will most likely be positional cloning.

The analysis of possible linkage disequilibrium in our families revealed no significant allelic association with any of the markers tested. Our results show that the underlying molecular defects leading to CNF in Finnish and non-Finnish populations seem to originate from mutations of the same CNF gene. AFP measurement in amniotic fluid and maternal serum cannot be performed before 16 weeks of pregnancy and may give false-negative results; indirect genetic analysis – available 4 weeks earlier – represents an additional prenatal diagnostic option in both Finnish and non-Finnish CNF-populations.

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Ask the expert*

Are there restrictions to vaccinating a child who has recovered from hemolytic uremic syndrome?

Key words: Hemolytic uremic syndrome – Immunization – Vaccination

In order to answer this question, it is necessary to review, briefly, our current understanding of the pathogenesis of hemolytic uremic syndrome (HUS). It has become increasingly clear that children with HUS can be separated into two broad groups on the basis of the presence of diarrhea (typical D+ HUS) and the absence of diarrhea (atypical D– HUS). It is also clear that diarrhea cannot be used alone to differentiate typical D+ from atypical D– HUS [1]. In contrast to patients with atypical D– HUS, those with D+ HUS rarely have recurrent episodes. In one reported instance, a second episode of HUS, and in another, a second episode of hemorrhagic colitis, have been associated with re-exposure to *Escherichia coli* 0157:H7 [2, 3].

There is abundant evidence that in a majority of patients with typical D+ HUS the causative organism is *E. coli* 0157:H7 and that the inciting factor is a shiga-like toxin, probably S-LT II [4–6]. There has been no evidence implicating any type of vaccine or immunization in the causation or pathogenesis of typical D+ HUS. Therefore there are no contraindications to immunizing or vaccinating children who have recovered from typical D+ HUS.

It is not as easy to give a definite answer for patients with atypical D– HUS, especially those who have recurrent episodes. Some of these patients appear to experience recurrences after minor "viral" infections, and after childhood illnesses in at least one case [7]. It is unwise to base a policy on one case, but I am unwilling to do anything that might precipitate another episode of HUS in a patient with atypical D– HUS. However, I think that it is important to weigh the benefits of immunizing and vaccinating such a patient against the potential, unproven costs. It is also important to consider other factors, such as the age of the patient, prior immunizations, and the risk of exposure to a particular illness. For example, it may not be necessary to give a booster shot of diphtheria, pertussis, and tetanus to a 6-year-old child, but it may be prudent to offer mumps vaccine to a boy or measles vaccine during an epidemic of measles.

In summary, I would not withhold immunizations from a patient with D+ HUS. I would think carefully before giving them to someone who has had atypical D– HUS, and I would be unwilling to give them to a person who has had a recurrence of atypical D– HUS.

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* The editors invite questions for this section