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EXCLUSION OF HOMOZYGOUS *PLCE1* (*NPHS3*) MUTATIONS IN 69 FAMILIES WITH IDIOPATHIC AND HEREDITARY FSGS

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Abstract

Focal and segmental glomerulosclerosis (FSGS) is the most common glomerular cause of end-stage kidney disease (ESKD). The etiology of FSGS has not been fully elucidated; recent results from the positional cloning of genes mutated in nephrotic syndromes are now beginning to provide insight into the pathogenesis of these diseases. Mutations in *PLCE1/NPHS3* were recently reported as a cause of nephrotic syndrome characterized by diffuse mesangial sclerosis (DMS) histology. One single family with a missense mutation had late onset of the disease that was characterized by FSGS. To further define the role of *PLCE1* mutations in the etiology of FSGS, we performed mutational analysis in 69 families with FSGS.

Results—A total of 69 families with 231 affected individuals were examined. The median age of disease onset was 26 years (range 1–66). Onset of ESKD was a median of 35.5 years. Seven variants leading to non-synonymous changes were found, of these, only two were new variants (exon4 c.1682 G>A R561Q, exon31 c.6518A>G K2173R). No known disease-causing mutations were identified in the families screened.

Conclusion—*PLCE1/NPHS3* mutations are not a cause of FSGS in this cohort. Absence of mutations in *PLCE1/NPHS3* in this study indicates that there are additional genetic causes of FSGS and that hereditary FSGS is a heterogeneous disease. Kindreds appropriate for genome-wide screening will be subjected to analysis to identify other genetic causes of FSGS.

Keywords

PLCE1 Mutation; FSGS; familial focal segmental glomerulosclerosis; familial nephropathy; genetics; hereditary

Introduction

Focal and segmental glomerulosclerosis is a clinicopathological entity that is characterized by the nephrotic syndrome and is often steroid resistant. Progression to ESKD is frequent. Histologically, the lesion is characterized by focal glomerulosclerosis or tuft collapse, segmental hyalinosis, occasionally IgM staining on immunofluorescence and effacement of foot processes on electron microscopy (1). It is responsible for 2–20% of all cases of ESKD

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in the United States and is the most common cause of glomerular disease in this subset (2,3). The incidence is higher in blacks than whites, with a striking difference in the age distribution pattern in blacks; the highest incidence of the disease occurring in the 40–49 year age group (2,3).

Current doctrine is that the primary defect in FSGS is in the filtration barrier of the glomerulus. The barrier is made up of fenestrated endothelium, the glomerular basement membrane and podocytes which have a slit diaphragm between their interdigitating foot processes. Disruption of the filtration barrier results in loss of permselectivity and albuminuria. The pathogenesis of FSGS has not been fully elucidated; however recent data from studies of familial cases reveal mutations in genes that encode the slit diaphragm and podocyte cytoskeletal proteins suggesting that FSGS is a primary disease of the podocyte. The first major breakthrough was the cloning of nephrin (*NPHS1*) as a cause of congenital nephrotic syndrome of the Finnish type (4). Since then, four additional genes have been identified, including podocin (*NPHS2*), actinin-α4 (*FSGS1*), transient receptor potential cation channel, type 6 (*TRPC6/FSGS2*), and CD2-associated protein (*FSGS3*) as causes of FSGS and hereditary nephrotic syndromes (5–8).

Mutations in phospholipase c epsilon-1 (*PLCE1/NPHS3*) were recently reported as a cause of early onset nephrotic syndrome that is characterized by histology of diffuse mesangial sclerosis (DMS) (9). The *PLCE1* gene is on chromosome 10q23. PLCε1 is a member of the phospholipase family of enzymes that catalyzes the hydrolysis of polyphosphoinositides to generate second messengers such as inositol-1, 4, 5 trisphosphate and diacylglycerol (9). These second messengers are involved in cell growth and differentiation (9). PLCε1 is expressed in the podocyte and the mechanism by which it causes DMS has not been fully elucidated. There is however *in vitro* data to suggest that it may act as a scaffolding protein in the glomerular slit diaphragm (9). All the mutations reported in children with DMS were loss of function mutations. Interestingly, two of the children in the original report had the histology of FSGS; in addition, they both had later onset disease and a missense mutation in contrast to children with DMS who all had early onset disease and truncating/loss of function mutations. These findings suggest that *PLCE1* mutations may have a role in the pathogenesis of FSGS either as the cause of the disease or at least as a modifier gene acting in concert with other mutations and environmental factors.

To further define the role of *PLCE1* mutations in the etiology and pathogenesis of familial FSGS, we performed mutational analysis in a world-wide cohort of 69 families with FSGS.

Methods

Clinical data

Institutional Review Board approval was obtained from Duke University Medical Center (Durham, NC, USA). Methods of subjects recruitment has been previously reported (11). Inclusion criteria in this study are 1) Diagnosis of biopsy-proven FSGS in at least two family members with multiple affected members. Additionally in cases of idiopathic FSGS, a diagnosis of biopsy-proven FSGS was required; 2) Exclusion of secondary causes of FSGS such as HIV infection, hepatitis and obesity; 3) Exclusion of mutations in *NPHS1, NPHS2, ACTN4* and *TRPC6*. Clinical data obtained included a complete family history; other associated morbidities, age at onset of disease and age at ESKD, urinalysis and serum creatinine were measured as appropriate.

Mutational analysis

Genomic DNA was extracted from whole blood using the Puregene kit. Mutational analysis was carried out by sequencing of both strands of all exons of *PLCE1* using exon flanking primers. All sequences were analyzed with the Sequencher software (Gene Codes Corp).

Data analysis

The clinical data and frequency of mutation and sequence variants were compared between the single and multi-generation families. All categorical data were compared by means of χ^2 test and continuous variable by student t test if they were normally distributed and Kruskal Wallis test for continuous data that are not normally distributed.

Results

Clinical phenotype

We studied 69 families with 231 affected individuals. They comprised ten families with only one person affected (14.5%), 19 families with two or more affected individuals in one generation (27.5%) and (58%) 40 families with two or more individuals in at least two generations. The kindreds with only one affected were classified as idiopathic (10/69 or 14.5%), two or more individuals in only one generation were classified as single generation (SG: 19/69 or 27.5%) and those with affected individuals in at least two generations were classified as multi-generation (MG: 40/69 or 58%). Kindreds with more than one affected individual (SG) in one generation were assumed to be autosomal recessive. Kindreds with affected individuals in more than one generation and male to male transmission (MG) were assumed to be autosomal recessive. None of the families are known to be consanguineous. The racial distribution, age at onset of disease and the age at ESKD was not different between the SG and the MG group (Table 1). At least one individual in 52.6% (10/19) of the SG families and 55% (22/40) of the MG families are known to have had a kidney transplant. Two families had one individual each with recurrence of FSGS in their renal allograft and both were in the MG group.

Mutation analysis

We found no new or previously documented disease causing mutations in any of the 69 families studied. We found eight non-synonymous changes in the 69 families (Table 2). Six of these changes are known single nucleotide polymorphisms (SNPs). Two heterozygous missense sequence variations (exon 4 c. 1682G>A R561Q and exon 31 c.6518A>G K2173R) found in two separate families are novel variants that have not been previously reported.

Discussion

The molecular etiology and pathogenesis of FSGS is still under investigation with new information being generated from positional cloning. Mutations in *PLCE1* were recently reported as a cause of early onset nephrotic syndrome characterized mainly by DMS histology pattern although two individuals were found to have FSGS, implying that mutations in this gene may be a cause of some cases of FSGS (9). In this study we performed mutation analysis in *PLCE1* in 69 families, 59 of the families have at least two or more affected individuals and only one affected individual in 10 families. Our cohort is therefore predominantly made up of families with FSGS inherited in a mendelian fashion which is likely due to a single gene defect. It is possible that the ten idiopathic cases may represent individuals with polygenic inheritance.

Two individuals from separate families had recurrence of FSGS in their renal allograft. One of them is from a large Central European family with 13 affected individuals spread across four generations while the other individual is from a North American family with affected individuals in two generations. The source of the kidney transplant from one person is unknown but the other individual had a living related kidney transplant. The disease recurrence rate in families with familial FSGS in this cohort is low when compared to the known recurrence rate for idiopathic FSGS (12). The low recurrence risk seen in familial cases in this series is however similar to what was previously reported in subjects with NPHS2 mutations, where it was found that the rate of recurrence of disease in renal allografts was considerably lower than in those without NPHS2 mutations (13). Possible explanations for disease recurrence in the two subjects in the present study could be because the defective gene and its product may not necessarily be localized to the kidney or the podocyte. Also the individual who received an LRD kidney may have obtained it from an obligate heterozygote who may have been asymptomatic at the time of the transplant. It is also possible that the two individuals are phenocopies with idiopathic FSGS and their disease may not be due to the genetic defects in the family.

We did not find disease causing homozygous or compound heterozygous mutations in any of the families studied. This is similar to a recent Dutch study that found no mutations in *PLCE1* in 19 cases of childhood-onset FSGS (14). In contrast, in a study of 57 families with autosomal recessive FSGS, Nevo *et. al* reported truncating and missense mutation in six families (15). All the affected families in the study had rapidly progressive disease leading to ESKD before the age of seven years. The reason for the difference between our findings and the observations in previous studies is not clear. It is possible that most of the families in our study have autosomal dominant disease because there are more than 2 generations affected with male-to-male transmission, however even in the subset with apparent autosomal recessive inheritance, we did not detect any mutation. The two individuals reported in the original description of *PLCE1* mutations are from a consanguineous Turkish family (9), the ethnic origin of the families from the Nevo *et. al* study is unknown. In our series, none of the families are known to be consanguineous and most of the families are from North America and Central Europe. It is therefore possible that *PLCE1* mutations are rare in this population. The other difference between our series and the two previous reports is the late onset of disease in our cohort, this finding will be in keeping with the initial observation that *PLCE1* may be important during glomerulogenesis by affecting capillary development and scaffolding in the slit diaphragm and will therefore cause early onset disease. We found two novel heterozygous variants in two different families (exon4 c. 1682G>A R561Q and exon31 c.6518A>G K2173R). Exon 4 of the gene encodes for the Ras binding domain of PLCε1 and exon 31 encodes for the C-terminal Ras-associating (RA) domain of the protein. Both domains are important for the ability of PLCε1 to function as an initiator and recipient of activated G protein signals (10). The two variants are conserved in evolution down to the zebrafish (R561Q change) and to drosophila (K2173R change). However, despite this impressive conservation in evolution, we did not find this change in all affected individuals and we also found the same change in some unaffected members of the families implying that these changes are unlikely to be a cause of disease. The amino acid change in the exon 4 variant is from arginine (a basic amino acid to glutamine a neutral amino acid) while the variant resulting in exon 31 change are both basic amino acids. Analysis of the amino acid substitution using the software developed by Sunyaev *et al* showed that the two amino acid substitutions are unlikely to be deleterious to the functions of PLCε1 (16).

In conclusion, we did not find *PLCE1* mutations in this cohort suggesting that the mutation in this gene is a very rare cause of FSGS in this population. Routine screening for mutations in individuals with hereditary autosomal dominant FSGS is therefore not warranted and

mutational analysis in this gene should probably be limited to disease with DMS histology. The absence of mutations in *PLCE1* and other known FSGS genes is an indication that there are additional genetic causes of FSGS. We are subjecting the families in the present study to a genome wide search to identify other causes of FSGS.

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Table 1

Clinical characteristics of 69 families with idiopathic and familial FSGS

Parameters	Idiopathic (n=10)	SG + (n=19)	MG+ (n=40)
Race (%)			
White	60.0	68.4	65.0
African American	30.0	21.1	30.0
Others	10.0	10.5	5.0
Age onset median (range) yrs	36.5 (16–45)	19 (2.5–49)	26 (1–66)
Age ESKD median (range) yrs	35 (17–45)	31.75 (6–50)	39.5 (10–63)
Proteinuria median (range) g/24hrs	5.2 (1–10)	5.8 (2.4–15)	2.5 (1–10)
Response to therapy	0/2 (0%)	0/4 (0%)	2/9 (22.2%)
Transplant	3/10 (30.0%)	10/19 (52.6%)	22/40 (55%)
Transplant recurrence	0/3 (0%)	0/10 (0%)	2/22 (9.1%)

SG+: Single generation MG+: Multi generation

Table 2

Non synonymous variants in *PLCE1* in 69 families with FSGS

Exon	Nucleotide Change	Amino acid change	SNP number
3	1405T>A	S469T	rs17508082
4	1643G>T	R548L	rs17417407
4	1682G>A	R561Q	Novel
5	1927G>T	A643T	ENSSNP7355814
20	4724 G>C	R1575P	rs2274224
24	5330C>T	T1777I	rs3765524
26	5780A>G	H1927R	rs2274223
31	6518A>G	K2173R	Novel