Mutational and Biological Analysis of α-Actinin-4 in Focal Segmental Glomerulosclerosis

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Mutations in the α-actinin-4 gene (ACTN4) cause an autosomal dominant form of focal segmental glomerulosclerosis (FSGS). A mutational analysis was performed of ACTN4 in DNA from probands with a family history of FSGS as well as in individuals with nonfamilial FSGS. The possible contribution of noncoding variation in ACTN4 to the development of FSGS also was assessed. Multiple nucleotide variants were identified in coding and noncoding sequence. The segregation of nonsynonymous coding sequence variants was examined in the relevant families. Only a small number of nucleotide changes that seemed likely to be causing (or contributing to) disease were identified. Sequence changes that predicted I149del, W59R, V801M, R348Q, R837Q, and R310Q changes were identified. For studying their biologic relevance and their potential roles in the pathogenesis of FSGS, these variants were expressed as GFP-fusion proteins in cultured podocytes. F-actin binding assays also were performed. Three of these variants (W59R, I149del, and V801M) showed clear cellular mislocalization in the form of aggregates adjacent to the nucleus. Two of these mislocalized variants (W59R and I149del) also showed an increased actin-binding activity. The I149del mutation segregated with disease; W59R was found to be a *de novo* mutation in the proband. A total of five ACTN4 mutations that are believed to be disease causing (three reported previously and two novel) as well as a number of variants with unclear contribution to disease now have been identified. The possibility that some of these other variants increase the susceptibility to FSGS cannot be excluded. ACTN4 mutations seem to account for approximately 4% of familial FSGS.

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Mutations in the α-actinin-4 gene (ACTN4) can cause a slowly progressive form of kidney dysfunction characterized by mild to moderate proteinuria, renal insufficiency with slow progression to ESRD, and a focal and segmental glomerulosclerotic pattern of tissue injury (1). Previously, we described three missense mutations in ACTN4 that co-segregate with disease in families with autosomal dominant inheritance. These mutations increase the binding of ACTN4 to actin filaments as well as alter their intracellular localization (1–3). The underlying disease mechanism is not entirely clear, but studies to date are consistent with a gain-of-function model (2,3). It is also clear that ACTN4 plays a critical and nonredundant role in the glomerulus, as ACTN4-deficient mice have a severe podocytopathy (4).

Focal and segmental glomerulosclerosis (FSGS) is a clinically and genetically heterogeneous entity. Mutations in NPHS2, a slit-diaphragm protein, cause a recessively inherited form of FSGS and/or nephrotic syndrome of widely variable age of onset and disease severity (5–7). Mutations in the TRPC6 channel cause an autosomal dominant form of FSGS phenotypically similar to the ACTN4-associated form of disease (8,9). Other loci for familial forms of disease also have been documented (10 and our unpublished data).

To define better the spectrum of disease-causing (and disease-associated) ACTN4 mutations, we performed mutational analysis of probands from 141 pedigrees with a familial pattern of inheritance of FSGS. We also analyzed DNA from 171 individuals with FSGS but without an apparent familial disease pattern. We examined the segregation pattern of the ACTN4 variants that we identified. In addition, we examined the F-actin binding properties and the cellular localization of these variants.

Materials and Methods

Clinical Ascertainment

Blood was obtained from individuals with FSGS and family members after informed consent was given in accordance with a protocol approved by the Institutional Review Board at Brigham and Women's Hospital. Clinical history and pedigree information was obtained from available family members. Genomic DNA was isolated from peripheral blood leukocytes using Qiagen (Valencia, CA) columns. When possible, urine protein excretion was assessed using a DCA 2000 microalbumin/creatinine analyzer (Bayer Inc., Elkhart, IN). Control samples were

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drawn from the Coriell Institute sample collection and from unaffected spouses in families with FSGS. Pedigree FS-XL is a shown in Figure 1.

Mutational Analysis

DNA from FSGS-affected probands was analyzed by means of bidirectional sequencing for variants in the ACTN4 gene. Sequencing was performed using PCR-amplified genomic DNA. High-throughput capillary sequencing instrumentation and Sanger dideoxy DNA sequencing were used for mutation detection. Verification of sequence alterations in probands, family members, and control subjects was done using matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry-based (Sequenom, San Diego, CA) single-nucleotide polymorphism (SNP) genotyping at the Harvard-Partners Core Genotyping Facility (primer sequences are available as Supplementary Data).

Site-Directed Mutagenesis

Site-directed mutagenesis was performed to insert the variants that were identified into a full-length human ACTN4 cDNA clone essentially as described previously (1). Two mutagenic oligonucleotide primers that contained the desired mutation, flanked by unmodified nucleotide sequence, were designed. Mutagenesis was performed with amplification reaction using the human ACTN4 cDNA template, mutagenic primers, and Pfu DNA polymerase. Clones then were selected and sequenced to verify and identify mutants. These wild-type and mutant clones were subcloned into the pBluescript SK and pEGFP-N1 vectors.

Actin Binding Experiments

We performed *in vitro* transcription and translation of mutant (W59R, I149del, R310Q, Q348R, V801M, and R837Q) and wild-type ACTN4 using a TnT Coupled Reticulocyte Lysate kit (Promega, Madison, WI) in the presence of 35S-methionine and plasmid DNA (1 μg). Electrophoresis on a 10% acrylamide gel and exposure of the dried gel to radiographic film confirmed the presence of the expected approximately 105-kD product from each reaction. We incubated labeled *in vitro* translated product (5 μl) from a 50-μl reaction in an 80-μl reaction that contained 10 mM Tris-HCl (pH 7.4), 100 mM KCl, 2 mM $MgCl_2$, 0.5 mM ATP, 0.2 mM DTT (conditions in which nonspecific protein binding to actin is minimized), 6 μM G-actin, and varying concentrations of purified unlabeled rabbit skeletal-muscle α-actinin (both obtained from Cytoskeleton, Denver, CO). We allowed actin to polymerize in this buffer for 60 min at room temperature. Reactions were performed both with and without the addition of 2.5 μM unlabeled α-actinin. Reaction mixes were centrifuged at 100,000 $\times$ *g* for 30 min at 18°C. Supernatants were removed for gel analysis, and pellets were resuspended in the initial 80-μl volume. A total of 30 μl of each supernatant and resuspended pellet were separated by SDS-PAGE on a 10% gel. The gel was dried and exposed to radiographic film. Intensity of the approximately 105-kD bands was quantified using the ImageJ Software (National Institutes of Health, Bethesda, MD). The amount of ACTN4 sedimenting was expressed as a percentage of the total detected in the pellet plus the supernatant.

Cellular Localization

Immortalized wild-type podocytes were generated and cultured as described previously (11). Plasmids (1 μg) were transfected into subconfluent undifferentiated podocytes using Fugene6 transfection reagent (Roche, Indianapolis, IN). Cells were fixed on coverslips after 48 h, stained with DAPI, and analyzed by fluorescence microscopy. Pictures were captured with a Spot-cooled CCD camera and processed with Adobe Photoshop 6.0 software.

Sequence Analysis

DNA sequence was analyzed using the Sequencher program (Gene Codes Corp., Ann Arbor, MI). For sequence alignments (Figure 2), we used multiple α-actinin protein sequences. GenBank sequences from human (ACTN4, U48734; ACTN1, X15804; ACTN2, NP_001094; ACTN3, M86407), mouse ACTN4 (AF093775), rat ACTN4 (AAD12064), *Xenopus* ACTN4 (AAH77918), Zebrafish (NP_955880), *C. elegans* (NP_506127), *Drosophila* (NP_477485), and *Dictyostelium* (DDB0001890) were retrieved from the National Center for Biotechnology Information web site and aligned using the CLUSTAL format for T-COFFEE program version 2.11 (Figure 2) (12). The computer programs SIFT and PolyPhen were used to help predict the effect of missense amino acid changes on protein function (13,14). These programs use sequence conservation data and nature of the amino acid change to predict the likely biochemical effect of a given mutation.

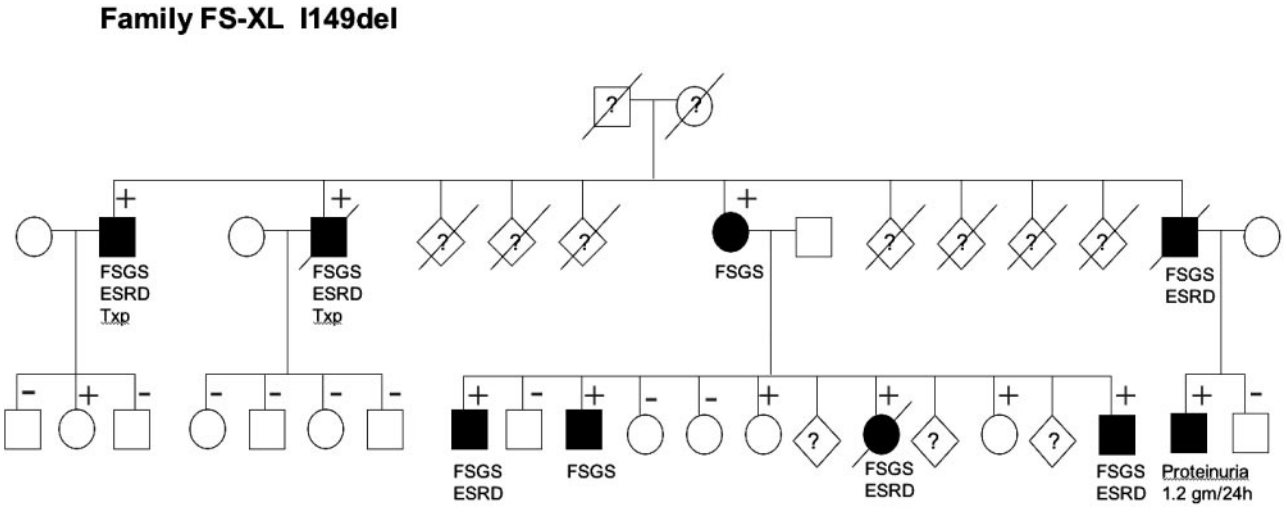


Figure 1. Pedigree figure illustrating phenotype and genotype segregation in family FS-XL. +, −, the presence or absence, respectively, of the I149del mutation; txp, recipient of renal transplant.

Figure 2. Sequence alignments of different α-actinin isoforms and α-actinins from different species illustrating the degree of conservation of altered amino acid residues. Alignments were performed using T-COFFEE (12).

SNP Genotyping

Genotyping of nucleotide variants was done using MALDI-TOF mass spectrometry-based (Sequenom) SNP genotyping. Genetic association between disease and ACTN4 SNP and haplotypes was evaluated using the HaploView program (15).

Results

We performed mutational analysis of probands from 141 pedigrees with a familial pattern of inheritance of FSGS. For this study, we decided to be fairly liberal in our classification of familial FSGS, as we believed that it was important to be sure to capture the full mutational and phenotypic spectrum of ACTN4-associated disease. We considered a proband to have familial FSGS when he or she was part of a family in which one or more individuals had a kidney biopsy showing (or reported to show) FSGS and at least one other individual with renal failure or proteinuria without a clear cause. A total of 42% of the families were of western European ancestry, 12% were of African ancestry, 16% were Hispanic, 4% were Middle Eastern, 2% were of Asian ancestry, and the remaining were of unclear ethnicity. In addition, we analyzed DNA from 171 individuals with FSGS but without an apparent familial disease pattern. We identified multiple ACTN4 sequence variants in individuals with FSGS. Several of these variants are predicted to alter the encoded amino acid sequence. These variants include amino acid changes A59R, I149del, R310Q, Q348R, A427T, V801M, and R837Q (Table 1). We examined the frequencies of these variants in control populations and in the families of FSGS probands whenever possible. We expressed variant GFP-tagged actinins in podocytes to examine cellular localization. We performed spin-down experiments to determine the effect of the amino acid substitutions on F-actin binding. The specific coding sequence variants identified are discussed below individually. Results are summarized in Table 1.

W59R

A W59R substitution was identified in the son of a Chinese mother and a father of western European ancestry. (We originally considered this to be a case of familial FSGS on the basis of ESRD and proteinuria in the maternal grandfather. Further evaluation of the grandfather's kidney biopsy showed that he had membranous nephropathy.) Neither parent was found to have this variant (confirmed by repeated sequence analyses), indicating that this was a *de novo* change in this individual. Nonpaternity was believed to be unlikely on the basis of consistent HLA typing data. This substitution was not identified in any individual with sporadic FSGS or in 326 control chromosomes.

The proband presented at the age of 5 yr with proteinuria

Table 1. Amino acid substitutions[a]

Amino Acid Change	Nucleotide Change	SIFT	PolyPhen	Cellular Localization	Altered F-Actin Binding	Allele Frequencies in Controls	Co-Segregation with Phenotype in Pedigree	Disease Causing or Contributing
A6T	C16G	Tolerated	Benign	ND	ND	ND	No	No
W59R	C175T	Affects function	Probably damaging	Abnormal	Yes	0	Yes	Yes
I149del	del(445-447)	NA	NA	Abnormal	Yes	0	Yes	Yes
K255E[b]	A763G	Affects function	Benign	Abnormal	Yes	0	Yes	Yes
T259I[b]	C776T	Affects function	Possibly damaging	Abnormal	Yes	0	Yes	Yes
S262P[b]	T784C	Affects function	Possibly damaging	Abnormal	Yes	0	Yes	Yes
R310Q	G929A	Tolerated	Possibly damaging	Normal	No	0.0074 (8/1084) controls 0.016 (3/192) sporadic FSGS	No	Probably not
Q348R	A1046G	Affects function	Possibly damaging	Normal	No	0	NA	Probably not
A427T	C1292G	Tolerated	Benign	ND	ND	ND	No	No
V801M	G2401A	Affects function	Benign	Abnormal	No	0.005 (5/961)	Yes	Probably not
R837Q	G2511A	Affects function	Benign	Normal	No	0	No	Probably not

[a]ND, not done; NA, not available.
[b]Previously reported (1).

(2.46 g protein/24 h) and a normal serum creatinine (0.4 mg/dl). His kidney disease progressed to ESRD within 3 yr, and he was started on peritoneal dialysis at 8 yr of age. At age 10, he received a kidney transplant from his father. He developed in the transplanted kidney recurrence of proteinuria (urine protein to creatinine ratio of 1.9 [normal <0.15]) that responded to treatment with plasmapheresis. Two years after transplantation, his kidney function declined; a biopsy of the transplanted kidney showed nonspecific interstitial fibrosis and tubular atrophy, but the possibility of recurrent FSGS in the transplant could not be excluded. In addition to FSGS, the proband has nonsyndromic bilateral high-frequency hearing loss.

When expressed in cultured podocytes, the ACTN4–W59R–GFP protein exhibited an abnormal localization pattern (Figure 3). The W59R–ACTN4 formed large aggregates located adjacent to the nucleus, whereas normal ACTN4 staining was almost completely absent. In actin-binding experiments using *in vitro* translated mutant ACTN4, the actin-binding activity of the mutant protein was increased (Figure 4) compared with the wild-type protein.

I149del

We identified a three-nucleotide deletion in ACTN4 in multiple members of one Colombian family. This alteration predicts the in-frame deletion of an isoleucine at position 149. We did not detect this deletion in any other individuals with FSGS or in 140 control chromosomes. I149del was present in 12 of 21 family members tested (Figure 1). Eight of the individuals who carry the I149del mutation have FSGS, two have proteinuria, and two have not shown clinical signs of kidney disease. The affected individuals developed evidence of kidney disease in young adulthood (early 20s to late 30s). Six of these individuals developed end-stage kidney disease, typically within 10 yr of initial diagnosis of kidney disease.

The biologic phenotype *in vitro* is similar to what we observed with the W59R mutation. Expression of the corresponding ACTN4–I149del–GFP fusion protein in mouse podocytes showed clear mislocalization comparable to the phenotypes seen with the W59R mutant (Figure 3) as well as in the previously reported variants (1,3). Actin-binding activity was increased (Figure 4) compared with wild-type ACTN4.

R310Q

We identified an ACTN4 R310Q substitution in probands of six of 141 families, four of whom are white, one of whom is western European/Native American, and one of whom is black. We found this variant in three of 175 individuals with sporadic FSGS (four of 350 chromosomes) and eight of 551 control individuals (eight of 1102 chromosomes). This variant did not co-segregate with disease in any obvious way within families. The six families with this variant were not large enough to perform meaningful calculations of nonparametric lod scores to assess the possibility that this polymorphism contributes weakly to disease susceptibility. The allele frequencies in probands of families with FSGS (0.021) and individuals with sporadic FSGS (0.0086) both are greater than the allele frequencies in control chromosomes assayed (0.0073). A two-sided Fisher exact test that was used to evaluate the significance of the approximately three-fold greater frequency in family probands compared with controls yields $P = 0.047$.

When expressed in cultured podocytes as a GFP-fusion protein, the R310Q variant shows a localization pattern similar to wild-type ACTN4. We did not observe GFP mislocalization in cells that were transfected with this variant, in contrast to what we observed with the variants discussed above. Consistent

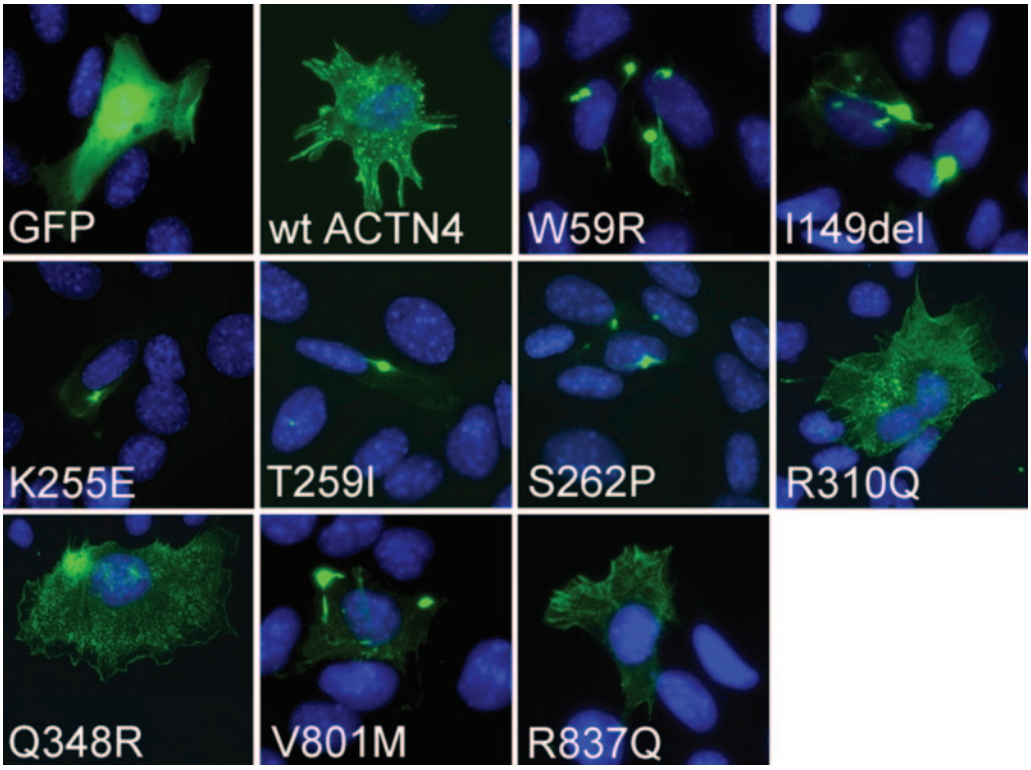


Figure 3. Cellular localization of α-actinin-4 (ACTN4) variants. GFP-tagged ACTN4 was expressed in immortalized podocytes and visualized by fluorescence microscopy. Nuclei are stained with DAPI. GFP alone and wild-type ACTN4 controls are shown as controls. The previously identified K255E mutation is shown as a positive control (1).

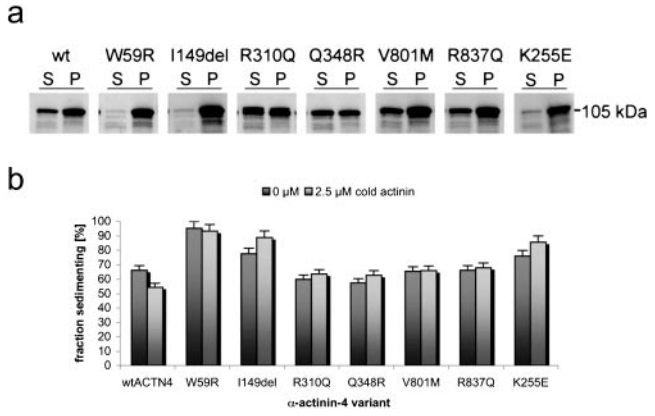


Figure 4. (a) Results of F-actin spin-down experiments for various ACTN4 variants. As described in Materials and Methods, radiolabeled ACTN4 was incubated with actin filaments followed by centrifugation. For each variant, the left lane shows supernatant and the right lane shows the resuspended pellet. Results using wild-type (wt) and K255E ACTN4 are shown as negative and positive controls. (b) Graphical representation of results of spin-down experiments. For each variant, the left bar indicates the percentage of labeled ACTN4 that is recovered from the pellet, and the right bar indicates the same percentage when 2.5 μm of cold α-actinin is added to the reaction before centrifugation, as per reference (1).

with the cell biologic phenotype, we could not detect any difference in the mutant protein's actin-binding properties compared with the wild-type protein. The Polyphen program predicts this R310Q substitution to be "possibly damaging," whereas the SIFT program predicts this substitution is be tolerated by the ACTN4 protein.

Q348R

We identified a Q438R substitution in one black woman with sporadic FSGS without a family history of kidney disease. This individual developed proteinuria at the age of 20 and was subsequently found to have FSGS. When expressed as GFP-fusion protein in cultured podocytes, the Q348R ACTN4 did not seem different from wild-type. We could not demonstrate any alteration in actin-binding activity in repeated actin spin-down experiments. This variant, which alters a highly conserved amino acid residue, was not identified in 1092 control chromosomes assayed. Both the SIFT and Polyphen programs suggest that this substitution may affect protein function.

V801M

We identified a V801M variant in probands of two families of western European descent. In one family, an FSGS-affected father and son both carried this variant. This variant was also found in an affected proband of a family with multiple affected members who were unavailable for genetic analysis. This variant was present in five of 961 control chromosomes assayed. The V801M variant shows altered cellular localization exhibit-

ing large aggregates in the cytoplasm resembling the structures seen with the W59R and the I149del mutant. In contrast, it does not exhibit a significantly altered actin-binding activity compared with the wild-type ACTN4. The SIFT program predicts that this substitution will affect protein function, whereas Polyphen predicts that it is a benign change. On the basis of the available information, we cannot rule out the possibility that this variant contributes to disease susceptibility.

R837Q

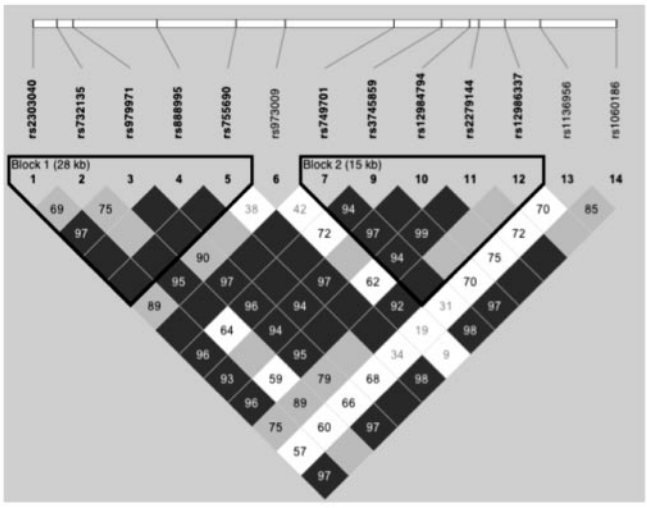
An R837Q variant was observed in probands of five families. This variant did not segregate with disease. When expressed as GFP-fusion protein in cultured podocytes, the R837Q ACTN4 did not seem different from wild type (Figure 3). We could not demonstrate any alteration in actin-binding activity in repeated actin spin-down experiments (Figure 4). We did not observe this variant in any of 88 sporadic FSGS samples (zero of 176 alleles) or in any of 166 control individuals (zero of 332 alleles). The SIFT program predicts this substitution to affect protein function, whereas Polyphen predicts that it is a benign change. On the basis of the available information, we cannot rule out the possibility that this variant contributes to disease susceptibility.

ACTN4 Polymorphisms

We genotyped several ACTN4 SNP in DNA from probands from 95 families with FSGS, 94 patients with sporadic FSGS, and 87 control individuals (control individuals were 57% western European and 43% black). We used the HaploView program to view ACTN4 haplotypes and to examine possible association between ACTN4 SNP genotype or haplotype and the presence of disease (15). We did not observe any strong associations between SNP genotype (either single SNP or haplotype block) and presence or absence of disease. Figure 5b shows χ^2 and P values for allele frequencies in patients and control individuals. The weakly significant P values observed for a small number of alleles likely represent chance associations. These data are summarized in Figure 5.

Haplotype blocks are shown (Figure 5a) as defined by regions of high linkage disequilibrium (16). The ACTN4 gene has two major haplotype blocks. Associations between block 1 haplotypes and disease showed marginally significant P values for one haplotype allele for each block, but, given the multiple hypotheses tested, these likely represent chance findings. We found no evidence for a major effect of any of these common SNP or haplotypes on susceptibility to FSGS. When the western European ancestry and African ancestry subgroups (patients and control subjects) were examined separately, the same block patterns were observed (although less well defined in the African ancestry group, likely a result of both shorter regions of linkage disequilibrium and smaller sample size). Similar weak associations with disease were observed with SNP shown in Figure 5b in the western Eu-

a



b

	SNP Name	Major Allele (in cases, controls)	Case, Control Major allele freq.	Chi Square	p value
Block1	rs2303040	T, T	0.688, 0.610	3.136	0.0766
	rs732135	C, C	0.950, 0.930	0.883	0.3474
	rs979971	C, C	0.683, 0.576	5.674	**0.0172**
	rs888995	C, C	0.807, 0.878	4.062	**0.0439**
	rs755690	A, G	0.538, 0.571	5.501	**0.0190**
	rs973009	A, A	0.886, 0.868	0.372	0.5419
Block2	rs749701	T, C	0.544, 0.564	5.457	**0.0195**
	rs3745859	C, C	0.631, 0.570	1.85	0.1738
	rs12984794	C, C	0.782, 0.871	5.914	**0.0150**
	rs2279144	C, C	0.645, 0.570	2.842	0.0918
	rs12986337	T, T	0.948, 0.918	1.817	0.1777
	rs1136956	T, T	0.738, 0.771	0.666	0.4144
	rs1060186	G, G	0.692, 0.732	0.899	0.3430

c

Block	Haplotype Frequency	Case, Control Haplotype freq.	Chi Square	p value
Block 1				
TCCCA	0.339	0.358, 0.299	1.837	0.1753
CCTCG	0.331	0.331, 0.304	3.964	**0.0465**
TCCTA	0.162	0.162, 0.182	3.218	0.0729
TCCCG	0.074	0.074, 0.069	0.408	0.5230
TTCCG	0.054	0.054, 0.048	0.997	0.3181
TCTCG	0.031	0.031, 0.028	0.275	0.6001
Block 2				
CTCTT	0.367	0.367, 0.343	2.808	0.0938
TCCCT	0.315	0.315, 0.325	0.594	0.4410
TCTCT	0.188	0.188, 0.215	5.264	**0.0218**
CCCCC	0.061	0.061, 0.051	1.898	0.1684
CCCCT	0.050	0.050, 0.043	1.113	0.2915

Figure 5. (a) Haplotype block structure defined by 13 single-nucleotide polymorphisms (SNP) at the ACTN4 locus, generated using the HaploView program (15) with haplotype blocks defined using confidence intervals as defined by Gabriel *et al.* (16). Dark gray, strong evidence of linkage disequilibrium; light gray, uninformative; white, strong evidence of recombination. (b) SNP assayed. SNP rs number, frequencies of major allele in patients and control subjects, and assessment of patient/control association by χ^2 analysis are shown. (c) ACTN4 haplotype blocks, frequencies of block alleles in patients and control subjects, and χ^2 analysis are shown.

ropean ancestry subgroup; no associations were observed in the African ancestry subgroup.

Discussion

We screened DNA from individuals with familial and sporadic FSGS for mutations in the ACTN4. We expressed suspicious variant in cells and performed F-actin binding assays with the variant proteins. In addition to the three disease mutations that we reported previously (1), we identified two mutations that we believe to be disease causing. We identified other missense changes with an unclear contribution to the FSGS phenotype.

Out of 141 families with familial FSGS or suspected familial FSGS, we identified a total of five mutations that we believe are definitely disease causing (two new and three previously reported). Four of these mutations are missense, and one is an in-frame amino acid deletion. All five occur in the actin-binding domain of the encoded protein. Although this number is too small to derive definitive conclusions regarding the mutation spectrum, the absence of any premature stop codons or frameshift mutations support the notion that the mechanism of disease is, at least in part, biologically dominant and "gain of function" in nature. Our conclusions regarding the contribution of a particular variant to disease are based on the nature of the changes (Table 1 and Figure 2) as well as altered F-actin binding and intracellular localization (Figures 3 and 4).

The clinical phenotype associated with the W59R variant is of particular interest. The affected individual developed disease at an earlier age than we have observed in other families with ACTN4 mutations. In addition, this individual developed recurrent proteinuria after transplantation, which responded to plasmapheresis. Although it is not clear why disease should recur in this individual's renal allograft (with a normal ACTN4 genotype), this is consistent with previously reported findings of posttransplantation disease recurrence in podocin-defective patients (17). We are not aware of the development of recurrent proteinuria or FSGS in any other case of ACTN4-associated disease.

We observed an increased frequency of the R310Q variant in patients with FSGS compared with control subjects. We are eager to see whether large-scale and better controlled population-based studies of genetic factors in the susceptibility to proteinuric kidney disease confirms this observation. We also identified the R837Q variant in probands of several families (but not co-segregating with disease) but not in those with sporadic FSGS or control subjects. Neither change was associated with biologic changes in protein behavior. A Q348R change that was observed in one patient with sporadic FSGS was not found in control subjects but did not alter protein localization or actin binding. We did not observe any compelling evidence that differences in ancestral ACTN4 haplotype conferred different susceptibilities to the development of FSGS.

What is the relationship between altered actin binding and altered protein localization and human disease? In the four large families in which we observed compelling genetic data in favor of gene variants causing disease, all variant proteins show both altered localization and altered actin binding. In the case of the W59R variant, this *de novo* mutation, not present in either biologic parent, is not found in control subjects and alters both actin binding and localization. Identification of additional large families with ACTN4 mutations would be helpful in determining whether in fact these biologic changes are always observed in disease-causing mutations.

The absence of any disease-associated mutations with either premature stop or frameshift mutations or loss of normal F-actin binding supports the hypothesis that disease is, at least in part, a result of a gain-of-function mechanism. Again, identification of additional disease-associated mutations will help strengthen (or refute) this hypothesis.

Our data suggest that ACTN4 mutations accounts for approximately 3.5% of familial FSGS and less than 1% of sporadic FSGS. TRPC6 mutations seem to account for a similar or slightly greater fraction of familial FSGS (8,9). Mutations in the podocin gene NPHS2 seem to account for a larger fraction of disease, on the order of 10% of familial disease in adults, and, in some studies, as much as 26% of all steroid-resistant nephrotic syndrome in pediatric populations (6,18). Thus, a considerable fraction of FSGS remains unexplained by mutations in known genes. In addition, although we are unable to demonstrate a clear role for more common variants in susceptibility to FSGS, we have identified some variants (Table 1 and Figure 5) that may deserve further examination in larger, well-controlled, population-based studies of kidney disease susceptibility.

Acknowledgments

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