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Mutations in the *INF2* gene account for a significant proportion of familial but not sporadic focal and segmental glomerulosclerosis

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Mutations in the inverted formin 2 gene (*INF2*) have recently been identified as the most common cause of autosomal dominant focal and segmental glomerulosclerosis (FSGS). To quantify the contribution of various genes contributing to FSGS, we sequenced *INF2* where all mutations have previously been described (exons 2 to 5) in a total of 215 probands and 281 sporadic individuals with FSGS, along with other known genes accounting for autosomal dominant FSGS (*ACTN4*, *TRPC6*, and *CD2AP*) in 213 probands. Variants were classified as disease-causing if they altered the amino acid sequence and if they were not found in control samples and in families segregated with disease. Mutations in *INF2* were found in a total of 20 of the 215 families (including those previously reported) in our cohort of autosomal dominant familial nephrotic syndrome or FSGS, thereby explaining disease in 9%. *INF2* mutations were found in 2 of 281 individuals with sporadic FSGS. In contrast, *ACTN4*- and *TRPC6*-related diseases accounted for 3 and 2% of our familial cohort, respectively. *INF2*-related disease showed variable penetrance, with onset of disease ranging widely from childhood to adulthood, and commonly leading to end-stage renal disease in the third and fourth decade of life. Thus, mutations in *INF2* are a more common, although still a minor, monogenic cause of familial FSGS when compared with other known autosomal dominant genes associated with FSGS.

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Glomerular disease, characterized by focal and segmental glomerulosclerosis (FSGS) histology, is a challenging disease to treat because of its frequent relapsing unremitting course and high rate of progression to end-stage renal disease (ESRD). For unclear reasons, FSGS has a rising incidence, now becoming a common cause of glomerular disease in both children and adults worldwide.[1–4] Although only a minority of those affected by FSGS have a family history of this lesion, the study of hereditary forms has helped inform our understanding of the molecular pathogenesis of FSGS. Mutations in *ACTN4*, *TRPC6*, and *CD2AP* are all rare causes of FSGS, although never quantified in the literature.[5–8] In contrast, several recent studies suggest that mutations in the inverted formin 2 gene (*INF2*) account for a significant proportion of hereditary cases.[9–11] INF2 belongs to the formin family, a group of heterogeneous actin-binding proteins that regulate a variety of cytoskeleton-dependent cellular processes.[12–16] Moreover, *INF2* has been implicated in individuals with Charcot–Marie–Tooth (CMT) disease, who manifest FSGS as part of this syndrome.[17]

We previously reported *INF2* as a cause of autosomal dominant FSGS in 11 of 93 families screened. Initial screening of the entire gene revealed disease-causing mutations only in exons 2–4, coinciding with the diaphanous inhibitory domain (DID). In this study, we expand on our initial report by mutational analysis of the DNA sequence encoding the DID of *INF2* in a total of 215 probands from autosomal dominant FSGS families and also in 281 individuals with apparent sporadic disease. Known autosomal dominant FSGS genes including *ACTN4*, *TRPC6*, and *CD2AP* were also screened in 213 probands for comparison.

RESULTS

INF2 exons 2, 3, 4, and 5 were sequenced by the Sanger method in the DNA belonging to 215 probands from autosomal dominant FSGS families and 281 sporadic cases to evaluate variation in the DID domain of the gene. Given the absence of mutations detected outside of this domain, based on our own experience and other published reports, we restricted mutational screening to these exons.[9–11,17] Thirteen

missense variants in 20 families that segregated with disease were identified, thereby explaining disease in 9% of our cohort with hereditary proteinuric renal disease (Tables 1 and 2). Individuals whose clinical status was defined as indeterminate given their young age were also found to have the mutations in some instances. Eleven of these families were reported previously. These tables summarize results from our entire cohort with *INF2*-related disease.[11] We also now include sporadic cases of FSGS and identify mutations in two individuals (Tables 1 and 3). Scores from PolyPhen-2 software analysis (http://genetics.bwh.harvard.edu/pph2) to predict the functional effects of missense *INF2* variants ranged from 0.789 to 1, predicting that *INF2* variants were possibly or probably damaging. No *INF2* variants were present in any of the 10,800 control chromosomes assayed or referenced in dbSNP, 1000 Genomes Project, or in the National Heart, Lung and Blood Institute's Exome Sequencing Project. All mutations affected highly conserved residues (Figure 1).

Some of the mutations identified in our cohort have been described before, whereas others are novel (Tables 2 and 3). Certain codons appear to be hotspots for mutations.[9,10,17–19] For example, 5 families have a mutation affecting arginine at the 218th position. Three of these families share the p.R218Q mutation, whereas two share the p.R218W mutation. None of these families are known to be related, and this mutation has been described by other groups as well. Mutations in the arginine at the 214th position are also frequently seen in our cohort and others; two of our families have an arginine to histidine mutation and one family has an arginine to cysteine mutation. Finally, two families share the p.S186P mutation but are not known to be closely related.

Although we believe that there are certain 'hotspots' for mutations, an alternative explanation is that these mutations could have been passed from a common ancestor. However, in family FG-JN, with the recurrent mutation p.R218Q, the mutation appears to have arisen *de novo* in an apparent founder individual within the pedigree. We do not have this individual's DNA sample, but several of his unaffected siblings share the haplotype on which the mutation is found in later generations (data not shown). These unaffected siblings do not harbor the mutation, thus suggesting that their affected sibling is the founder.

Table 1 | Percentage of families and sporadics affected by *INF2*-related kidney disease in the current cohort compared with other groups reported in the literature[9,10]

	Barua *et al*	Boyer *et al*[9]	Gbadegesin *et al*[10]
Total number of families with *INF2* mutations	20	9	8
Total number of families tested	215	54	49
Percentage of families with *INF2* mutations	9	17	16
Number of sporadics with *INF2* mutations	2	1	0
Total number of sporadics tested	281	84	31
Percentage of sporadics with *INF2* mutations	0.7	1	0

Abbreviation: INF2, inverted formin 2 gene.

Table 2 | List of *INF2* heterozygous missense mutations by family and *in silico* protein function prediction according to Polyphen-2 software

Family ID	Exon number	Nucleotide change	Amino acid change	Polyphen-2 prediction	Polyphen-2 score	Found in other cohorts?	Previously reported?
FG-BR	4	c.556T>C	p.S186P	Probably damaging	0.988	Y	Y[11]
FG-DM	4	c.593T>G	p.L198R	Probably damaging	0.995	Y	Y[9]
FG-EA	4	c.652C>T	p.R218W	Probably damaging	1	Y	Y[11]
FG-EF	4	c.641G>A	p.R214H	Probably damaging	1	Y	Y[9,10]
FG-EP	2	c.125T>C	p.L42P	Probably damaging	0.995	N	Y[11]
FG-ER	4	c.556T>C	p.S186P	Possibly damaging	0.789	Y	Y[11]
FG-FG	4	c.641G>A	p.R214H	Probably damaging	1	Y	Y[9–11]
FG-GY	4	c.550G>A	p.E184K	Probably damaging	0.999	Y	Y[10]
FG-HT	3	c.472C>G	p.H158D	Probably damaging	1	N	N
FG-JN	4	c.653G>A	p.R218Q	Probably damaging	1	Y	Y[9–11]
FG-JY	4	c.640C>T	p.R214C	Probably damaging	1	Y	N[9,10]
FG-KM	2	c.217G>A	p.G73S	Probably damaging	0.999	N	N
FG-KQ	4	c.653G>A	p.R218Q	Probably damaging	1	Y	N[9–11]
FG-LL	4	c.542T>G	p.V181G	Probably damaging	0.989	N	N
FG-LP	4	c.529C>T	p.R177C	Probably damaging	1	N[a]	N[9,10]
FG-LW	4	c.653G>A	p.R218Q	Probably damaging	1	Y	N[9–11]
FG-LY	3	c.451T>C	p.C151R	Probably damaging	1	N	N
FG-ME	4	c.652C>T	p.R218W	Probably damaging	1	Y	N[9–11]
FS-B	4	c.658G>A	p.E220K	Probably damaging	0.999	N	Y[9,18]
FS-V	2	c.242T>C	p.L81P	Probably damaging	0.995	N	N[11]

Abbreviations: FSGS, focal and segmental glomerulosclerosis; *INF2*, inverted formin 2 gene; N, no; Y, yes.
Mutations that were found in other families, either in our cohort or published in the literature, are indicated. Families that were reported in our original INF2 discovery paper published in 2009 are also shown.[11] Alterations in nucleotide and amino acid sequence are reported using the following NCBI RefSeq accession numbers: INF2 – NM_022489 and NP_071934.
[a]This residue has been mutated in other families with FSGS but to a different amino acid.