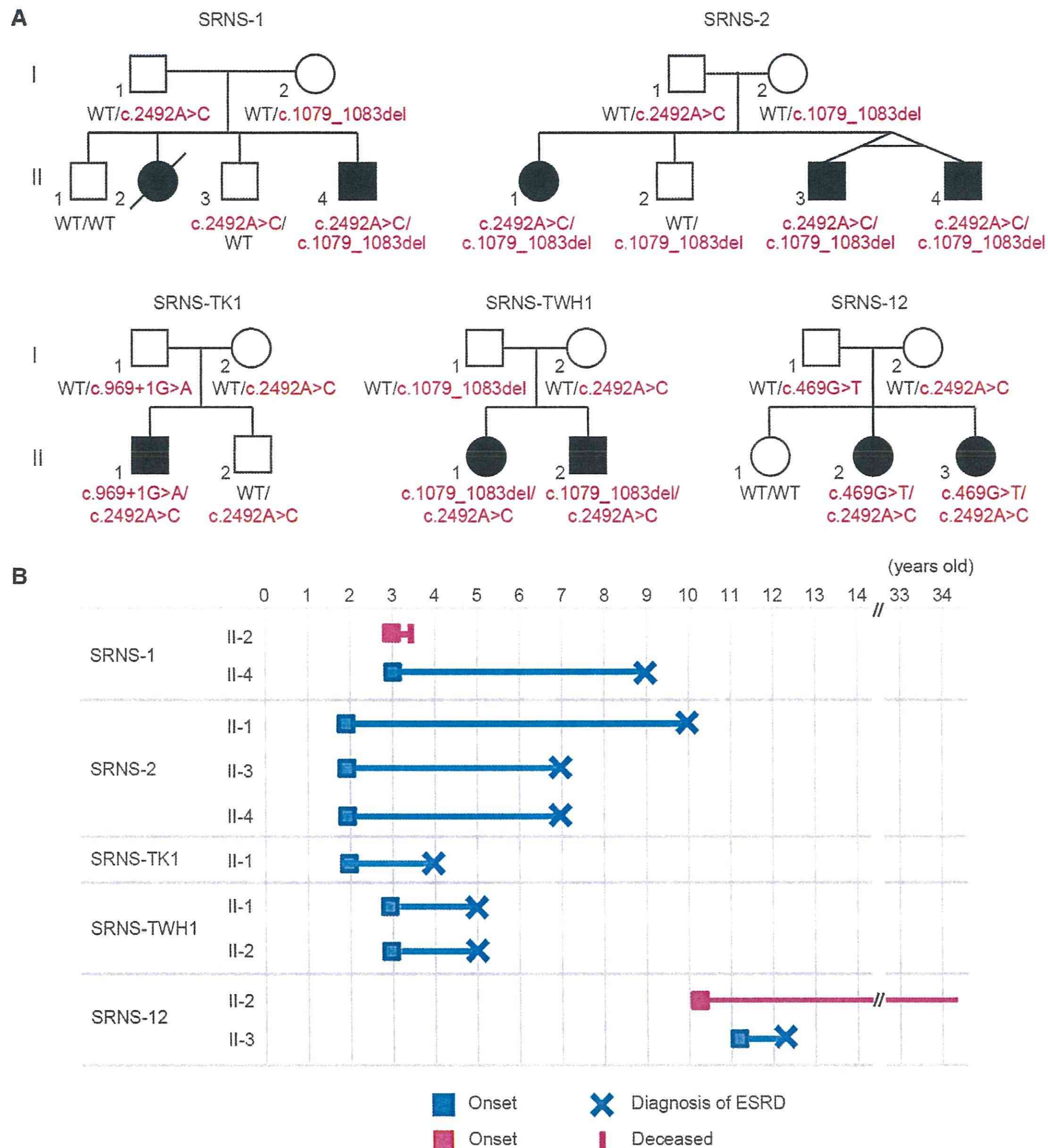

Figure 1. Genetic Analysis and Clinical Course of Early-Onset SRNS in Affected Individuals with *NUP107* Mutations
(A) Familial pedigrees and *NUP107* mutations. Mutant alleles are colored in red. WT indicates the wild-type allele. Filled and unfilled symbols represent affected and unaffected members, respectively.
(B) Clinical course of the affected individuals. The onset of renal symptoms and diagnosis of ESRD are represented by squares and crosses, respectively. Blue and red horizontal bars indicate the period leading to ESRD and the period before completed ESRD, respectively. SRNS-1 II-2 died from a viral infection before the advent of ESRD.

evidence strongly suggests that biallelic *NUP107* mutations could lead to autosomal-recessive SRNS.

A Common Haplotype Harboring c.2492A>C

Interestingly, all affected individuals carry c.2492A>C heterozygously. To determine whether c.2492A>C was derived from an ancestral chromosome, we constructed the haplotype in all families by using informative microsatellite markers and SNPs. We confirmed that a 412-kb haplotype was shared by all five families (Figure S6). Considering the extreme rarity of c.2492A>C in different whole-exome databases, c.2492A>C is likely to be specific to East Asians.

Clinical Characterization of NUP107-Related SRNS

Noticeably, the clinical course of affected individuals with *NUP107* mutations was similar (Figure 1B and the supplemental note). In brief, the four families consistently showed early-onset SRNS whereby NS first manifested itself at age 2–3 years and ESRD became evident before age 10

Table 1. Clinical and Genetic Summary of SRNS-Affected Families Harboring *NUP107* Mutations

Family	Individual	Mutation	Age at Onset (Years)	Age at Diagnosis of ESRD (Years)	Treatment	Histology (Subtype, Age in Years)
SRNS-1[a]	II-2[b]	ND	3	NA	Pred	FSGS (NOS, 3)
	II-4	c.[1079_1083del];[2492A>C]	3	9	Pred, CyA, CPA	FSGS (NOS, 3)
SRNS-2[a]	II-1	c.[1079_1083del];[2492A>C]	2	10	Pred, CPA	MCNS (NOS, 2), FSGS (NOS, 4)
	II-3	c.[1079_1083del];[2492A>C]	2	7	Pred	MCNS (2)
	II-4	c.[1079_1083del];[2492A>C]	2	7	Pred	FSGS (NOS, 2)
SRNS-TK1	II-1	c.[969+1G>A];[2492A>C]	2	4	Pred, CyA, CPA	FSGS (NOS, 2)
SRNS-TWH1	II-1	c.[1079_1083del];[2492A>C]	3	5	Pred, ARB, PP	FSGS (collapsing, 3)
	II-2	c.[1079_1083del];[2492A>C]	3	5	Pred, CyA, ARB	FSGS (collapsing, 3)
SRNS-12[a]	II-2	c.[469G>T];[2492A>C]	10	NA	ARB	ND
	II-3	c.[469G>T];[2492A>C]	11	12	Pred, ARB	FSGS (NOS, 11)

Abbreviations are as follows: ARB, AT II receptor blocker; collapsing, collapsing variants; CPA, cyclophosphamide; CyA, cyclosporine A; ESRD, end-stage renal disease; FSGS, focal segmental glomerulosclerosis; MCNS, minimal-change nephrotic syndrome; NA, not applicable; ND, not determined; NOS, non-specific type; PP, plasmapheresis; Pred, prednisone.
[a]These families appear in a previous report by Kitamura et al.[12]
[b]This individual died from a viral infection at the age of 3 years.

years. One family (SRNS-12) showed an exceptionally late onset of NS, which appeared after 10 years of age, and renal function has been relatively preserved at the current 34 years of age. Renal biopsies revealed histopathological FSGS in all affected individuals (Figure 2, Table 1, and Figure S7). Depletion of NUP107 was shown to lead to apoptosis in eukaryotes,[20,27] and we observed apoptotic changes in the renal biopsy samples from SRNS individuals (SRNS-TWH1 II-1 and II-2) with *NUP107* mutations. Cells with the characteristic morphological features, such as nuclear shrinkage and fragmentation, were occasionally found in the glomeruli and renal tubules (Figure S8). Some of these cells could be TUNEL positive (apoptotic), although we failed to recognize TUNEL-positive cells in the glomeruli of the few biopsied specimens, given that only ten glomeruli were observed (data not shown). Among them, five individuals underwent renal transplants and have experienced no recurrence of SRNS to date. Additionally, none of them showed neurological phenotypes.

NUP107 Function and *NUP107* Expression in Humans

NUP107 is an essential component of the NPC, which is one of the largest protein complexes (~125 MDa in vertebrates) in eukaryotes and comprises ~30 nucleoporins embedded in the nuclear envelope.[28,29] It facilitates the efficient transfer of macromolecules between the nucleus and cytoplasm in a highly selective manner and plays pivotal roles in the nuclear framework and gene expression.[28,30–33] Although some nucleoporins have tissue specificity,[34] *NUP107* and NUP107 are ubiquitously expressed as the core gene and the essential scaffold protein, respectively, of the NPC.[29,35–37] As the results of the TaqMan expression assay show, *NUP107* is expressed ubiquitously in most human fetal and adult tissues, including the kidney (Figure S9). To evaluate the physiological relevance of NUP107 in human podocytes, we examined the intracellular localization of NUP107, along with WT1 (a podocyte-specific transcription factor[38]) and Ezrin (a marker protein for apical domains of epithelial cells[39]), in human podocytes. Confocal microscopy demonstrated that NUP107 co-localized with WT1 and was distributed in a speckle-like pattern in the nuclei of human podocytes surrounding the glomerular capillary tufts (Figure S10). In addition to podocytes, most other cell types showed a similar staining pattern for NUP107. These data suggest that NUP107 has an important function for renal filtration in human podocytes. A direct link between NUP107 and renal disease has never been shown, but *NUP107* knockdown in HeLa cells altered the localization of ELYS, and this affected the proper localization of lamin A/C,[19] an alteration in which caused FSGS.[40]

Effect of the Common NUP107 p.Asp831Ala Substitution on the Structure of the Protein and Its Binding to NUP133

To evaluate the effect of p.Asp157Tyr and p.Asp831Ala substitutions from a structural viewpoint, we mapped the variant positions on the crystal structure of the yeast Sec13-Nup145C-Nup84 complex (PDB: 3IKO),[41] which is analogous to the human SEC13-NUP96-NUP107 complex (NUP96 is the C-terminal half product of *NUP98* [GenBank: NM_016320.4; MIM: 601021], processed after translation[42,43]) and the human NUP107-NUP133 complex (PDB: 3CQC).[14] Asp157 is predicted to reside on the surface of the protein, suggesting that the p.Asp157Tyr substitution does not affect the folded structure of NUP107 (Figure S11). However, because this protein interacts with many other proteins,[44] the possibility that the p.Asp157Tyr substitution might impair these interactions cannot be excluded, although no such changed

Table 2. *NUP107* Mutations in Affected Individuals with Early-Onset SRNS

Mutation	Amino Acid Change	PolyPhen-2	PyloP	MutationTaster	Grantham	EVS	ExAC	HGVD	In-House Exomes[a] (n = 575)
c.469G>T	p.Asp157Tyr	0.712	2.84	0.998403	160	0	0	0	0
c.969+1G>A	splice site	NA	NA	NA	NA	0	0	0	0
c.1079_1083delAAGAG	p.Glu360Glyfs*6	NA	NA	NA	NA	0	0.0000083	0	0.0008696
c.2492A>C	p.Asp831Ala	1.000	1.952	0.99995	126	0	0	0.0013587	0

Mutations were annotated according to *NUP107* cDNA (GenBank: NM_020401.2). Abbreviations are as follows: EVS, NHLBI Exome Sequencing Project Exome Variant Server; HGVD, Human Genetics Variation Database (the public exome database of the Japanese population).
[a]In-house exome database of Japanese control individuals.

interaction for this particular variant site has been reported. Because the Asp831 side chain forms hydrogen bonds with the Arg842 side chain, the p.Asp831Ala substitution is considered to disrupt these hydrogen bonds. To evaluate the effects of this variant on the structure of NUP107, we performed MD simulations for wild-type and altered NUP107 in solution. In this substitution, a region around the variant site and a region involved in interactions with NUP133 (amino acid residues 881–890) both showed more fluctuations than did those same regions in the wild-type protein (Figure S12). This NUP133-interacting region is considered to be structurally correlated with the variant site through van der Waals contacts (Figure S12B). The results from the MD simulations suggest that the p.Asp831Ala substitution impairs the molecular interaction between NUP107 and NUP133.

Impaired Function of the Altered NUP107

Because NUP107 interacts with NUP133 via its C-terminal tail,[14] we investigated the mutational effects on the protein-protein interaction between NUP107 and NUP133 in vitro. We used an in vitro pull-down assay with recombinant proteins produced in a wheat germ cell-free system to determine the contribution of the C-terminal region of NUP107. Consistent with a previous report,[14] the altered NUP107 that lacked a third of the C-terminal region (amino acids 645–925) did not bind to NUP133 as tightly as wild-type NUP107 under equilibrium conditions (Figure S13). Likewise, two truncated NUP107 proteins with extensively shorter C termini (p.Asp324* and p.Glu360Glyfs*6) also showed weaker binding to NUP133. Notably, a p.Asp831Ala protein with an altered C terminus exhibited significantly reduced binding to NUP133, whereas a p.Asp157Tyr protein with an altered N terminus retained full binding activity (Figure 3A). Wild-type GFP-fused NUP107, which was transiently produced by a mammalian expression vector, was bound to endogenous NUP133 in HeLa cells, and the p.Asp831Ala protein was also bound to NUP133 but weakly in comparison to the wild-type (Figure 3B). Observation of the intracellular localization of altered GFP-NUP107 indicated that the two truncated proteins were distributed mainly in the cytoplasm, whereas the wild-type protein was clearly localized in the nuclear envelope (Figure 3C). The p.Asp831Ala altered protein was localized in the nuclear envelope and cytoplasm (Figure 3C). These results are consistent with the impaired interaction observed between the altered NUP107 and NUP133.

Zebrafish with *nup107* Knockdown Have Glomerular Abnormalities Mimicking SRNS

Reportedly, zebrafish with homozygous *nup107* mutations and morphants with *nup107* knockdown produced with anti-sense MOs each similarly showed a thin pharyngeal skeleton, unfolded intestine, and loss of swim bladder and died on days 5 and 6.[20] However, the specific renal phenotype was not investigated. Therefore, we injected the *nup107*-TB MO or *nup107*-SB MO to create an in-frame (15-bp) deletion at exon 24 to mimic the commonly shared missense mutation (c.2492A>C [p.Asp831Ala]) and then carefully observed the renal phenotype in vivo (Figures S14 and S15). As reported previously,[20] neither of the zebrafish morphants developed edema until they died at around days 5 and 6 (Figure S14A). Furthermore, we sought to identify the glomerular filtration impairment in knockdown zebrafish (*nup107*-TB MO) but did not observe any traces of recognizable protein leakage in glomeruli at 96 hpf (data not shown). Although zebrafish might not be the best animal model for generating renal phenotypes, in a microscopic section of the *nup107*-SB morphant, we were able to find supportive findings in that the glomeruli were generally underdeveloped and showed hypoplastic or poorly organized capillary vessels and mesangial regions (Figures S14C–S14E). Electron microscopy revealed abnormally shaped foot processes and collapse of the capillary lumen in both morphants (Figures S14F–S14K and S16). Because these observations are similar to those from humans with FSGS, the zebrafish morphants might reflect the renal changes caused by the *NUP107* mutation.

Unchanged NPC Localization in Lymphoblastoid Cells from Affected Individuals with *NUP107* Mutations

Reportedly, NUP107 depletion results in decreased or absent NPCs.[29,36] However, a lymphoblastoid cell line derived from affected individuals showed no apparent NPC loss or abnormality by immunohistochemistry analysis (data not shown), which indicates that some residual

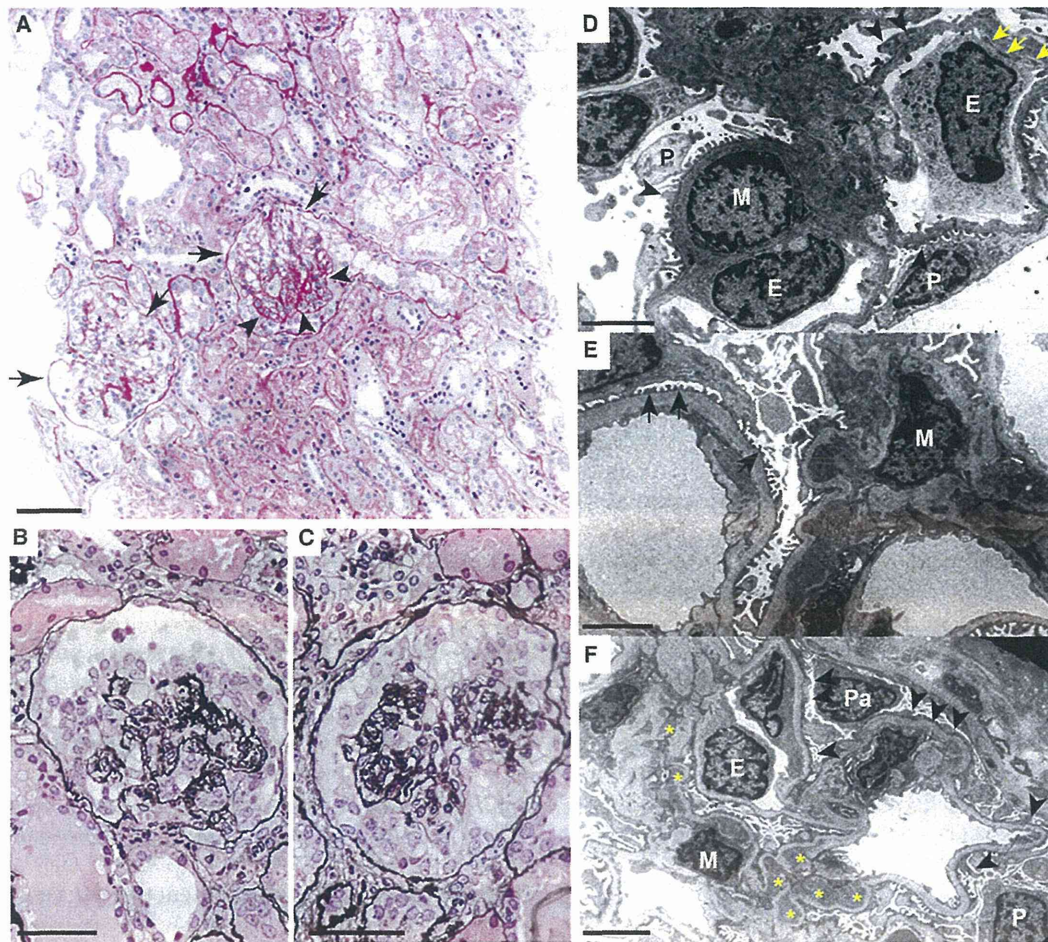

Figure 2. Kidney Histopathology of Affected Individuals with Biallelic *NUP107* Mutations
(A–C) Light micrographs of kidney biopsy specimens from SRNS-TWH II-1. (A) A low-power view (periodic acid-Schiff stain, 100× magnification) of two representative abnormal glomeruli (arrows). Half of the glomerulus is sclerosed (arrowheads). (B and C) Enlarged images (periodic acid methenamine silver stain, 400× magnification) show the collapse of glomerular tufts with hypertrophy and hyperplasia of the glomerular epithelial cells that fill the urinary space. Tubular injury accompanying atrophy of epithelia and interstitial fibrosis is noted.
(D–F) Electron micrographs of biopsy specimens from SRNS-2 II-1 (D), SRNS-2 II-3 (E), and SRNS-2 II-4 (F). Effacement of podocyte foot processes and some mesangial expansion with sub-endothelial electron-dense deposits are apparent. The thickness of the glomerular basement membrane appears normal and shows no evidence of splitting, lamellation, or fragmentation, thereby excluding the possibility of a primary basement-membrane defect. Accumulation of storage materials and dysmorphic mitochondria were not found in the podocyte cytoplasm. Abbreviations are as follows: E, endothelial cell; M, mesangial cell; P, podocyte; Pa, papillary epithelia. Arrowheads indicate effacement of podocyte foot processes, yellow arrows represent electron dense deposits, black arrows show flattened podocyte foot processes, and yellow asterisks show paramesangial deposits.
Scale bars represent100 μm (A), 40 μm (B and C), 2 μm (D and E), and 5 μm (F).

functions of altered NUP107 might persist in the cells of affected individuals, at least under non-stressful conditions. NUP107 is an essential scaffold protein in the NPC, a structure that is evolutionary conserved from yeast to vertebrates.[29,36] Therefore, in the null state, *NUP107* mutants might be lethal in humans.

Discussion

In this study, we have shown that biallelic *NUP107* mutations cause early-onset SRNS in humans. Affected individuals with *NUP107* mutations usually developed SRNS at 2–3 years of age and progressed to ESRD before 10 years of age but experienced no recurrence of the disease after renal transplantation. How do *NUP107* mutations cause a glomerular phenotype in humans? This might be partly explained by the specific properties of podocytes, which are highly differentiated with a unique architecture (foot processes and slit membranes).[45,46] In affected individuals with *NUP107* mutations, insufficient NUP107 function could cause immature and/or hypoplastic podocytes, or at least functionally impaired podocytes that are progressively destroyed by

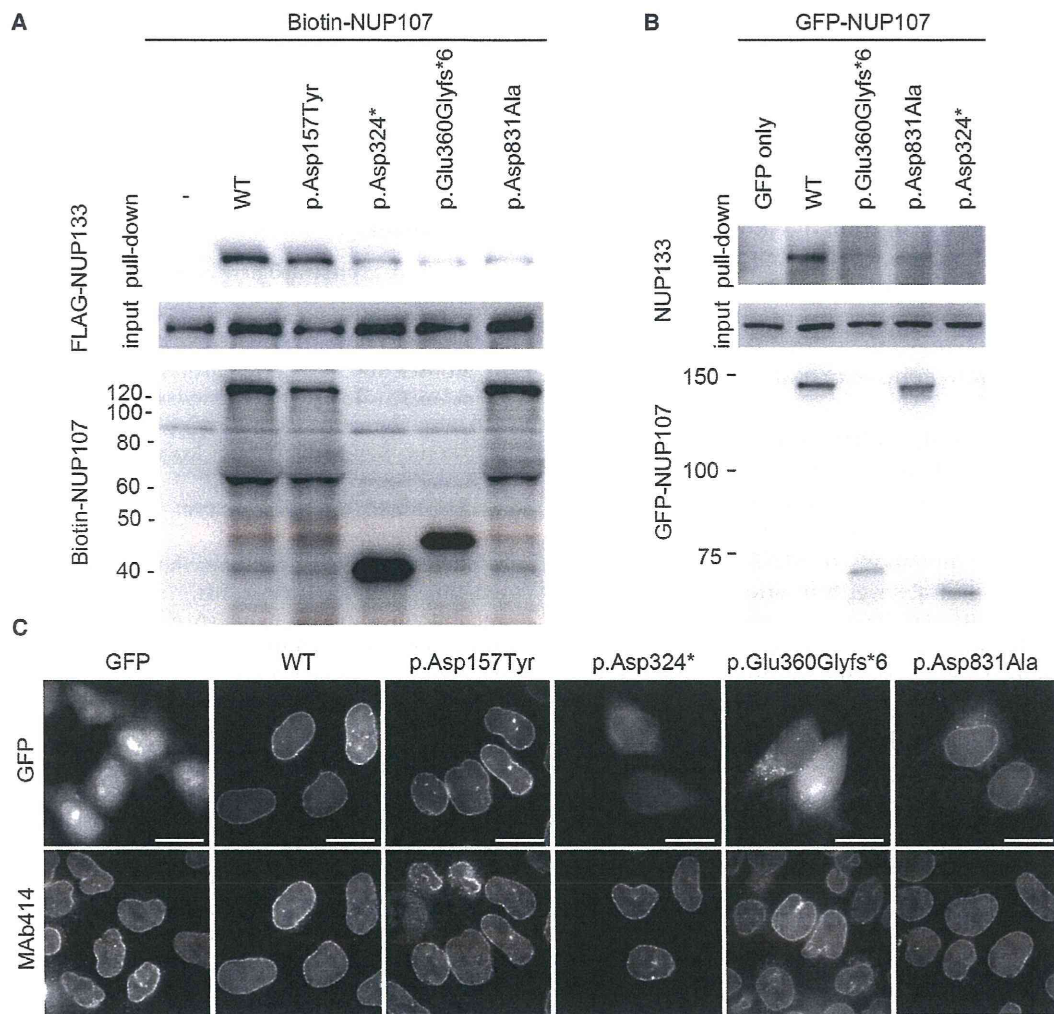

Figure 3. Decreased Intermolecular Interactions between NUP107 and NUP133
(A) In vitro protein-protein binding assay of altered NUP107 with NUP133. The FLAG-tagged NUP133 mixed with biotinylated altered NUP107 proteins was subjected to a pull-down assay with streptavidin magnetic beads. The bound proteins were separated by SDS-PAGE and then detected with an anti-FLAG antibody or with streptavidin-horseradish peroxidase. The corresponding protein inputs are shown in the middle and bottom panels.
(B) Evaluation of the interaction between NUP107 and NUP133 with the use of wild-type NUP107 and its alterations. Wild-type GFP-NUP107 or its alterations were transiently produced in HeLa cells and precipitated with an anti-GFP antibody. The NUP107-NUP133 interaction was analyzed via immunoblotting using the antibodies indicated.
(C) Subcellular localization of NUP107 or its alterations. For visualizing localization of altered or wild-type GFP-NUP107 in HeLa cells, the cells were fixed and stained with a MAb414 antibody recognizing the NPC on the nuclear envelopes. Scale bars represent 20 μm.
The following abbreviation is used: WT, wild-type.

increased filtration pressure after birth. Interestingly, nuclear-envelope proteins, including NPCs, are closely associated with mechanotransduction signaling,[47,48] and mechanical stretching decreases podocyte proliferation and cell-body size by reorganizing the actin cytoskeleton in vitro.[49,50] Thus, increased post-natal capillary pressure leading to mechanical stretching of vulnerable podocytes might accelerate glomerulus damage. Furthermore, mature podocytes do not regenerate.[51,52] Thus, the core pathological condition of SRNS caused by *NUP107* mutations is a structural abnormality, which correlates well with the early SRNS onset in childhood, its steroid resistance, and its lack of post-transplant relapse (Figure S17).

Recently, a homozygous missense mutation (c.303G>A [p.Met101Ile]) was reported in an affected individual who is from a consanguineous family and presents with global developmental delay and early-onset FSGS.[53] However, none of our affected individuals with *NUP107* recessive mutations show neurological impairment. Additional genetic factors might be involved in the neurological symptoms of the consanguineous family. Alternatively, different mutations could cause an additional neurological phenotype. This mutation has been suggested to lead to

abnormal splicing (and possibly a nearly null function), although no direct evidence has been shown.[53] As for p.Asp157Tyr, we could not find direct evidence of its functional impairment experimentally. However, it could be a hypomorphic variant; if so, this might explain the milder phenotype in the SRNS-12 family, who carries both missense mutations (c.469G>T [p.Asp157Tyr] and c.2492A>C [p.Asp831Ala]). Thus, it is possible that the residual NUP107 function left by missense mutations (including c.469G>T [p.Asp157Tyr]) is related to the late onset age and/or milder severity of the disease. It is intriguing that mutations in *NUP107*, which encodes an essential nucleoporin of the NPC, lead to a kidney-specific disease in humans.

In summary, biallelic *NUP107* mutations cause early-onset SRNS for which renal transplantation is the only effective treatment. Access to genetic information is useful for proper clinical management of NS. Therefore, screening *NUP107* mutations in SRNS individuals with broad ranges of clinical severity is strongly encouraged. Furthermore, we did not identify the genetic cause in six pairs of affected siblings and seven single affected individuals in our cohort, which implies a heterogenetic etiology for early-onset SRNS. Further research is necessary to uncover the whole picture of this type of SRNS.

Supplemental Data

Supplemental Data include a supplemental note, 17 figures, and 8 tables and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2015.08.013.

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Web Resources

The URLs for data presented herein are as follows:

1000 Genomes FTP site, ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/technical/reference/README.human_g1k_v37.fasta.txt
ExAC Browser, http://exac.broadinstitute.org/
Genome Analysis Toolkit, http://www.broadinstitute.org/gatk
HGVD, http://www.genome.med.kyoto-u.ac.jp/SnpDB/
NHLBI Exome Sequencing Project Exome Variant Server, http://evs.gs.washington.edu/EVS/
Novoalign, http://www.novocraft.com
OMIM, http://www.omim.org
PDB, http://www.rcsb.org/pdb/home/home.do
Picard, http://picard.sourceforge.net
RefSeq, http://www.ncbi.nlm.nih.gov/refseq/
UCSC Genome Browser, https://genome.ucsc.edu/

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ORIGINAL ARTICLE

End-stage renal disease in Japanese children: a nationwide survey during 2006–2011

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Abstract

Background End-stage renal disease (ESRD) in children is considered a rare, but serious condition. Epidemiological and demographic information on pediatric ESRD patients around the world is important to better understand this disease and to improve patient care. The Japanese Society for Pediatric Nephrology (JSPN) reported epidemiological and demographic data in 1998. Since then, however, there has been no nationwide survey on Japanese children with ESRD.

Methods The JSPN conducted a cross-sectional nationwide survey in 2012 to update information on the incidence, primary renal disease, initial treatment modalities, and survival in pediatric Japanese patients with ESRD aged less than 20 years during the period 2006–2011.

Results The average incidence of ESRD was 4.0 per million age-related population. Congenital anomalies of the kidney and urinary tract were the most common cause of ESRD, present in 39.8 % of these patients. In addition, 12.2 % had focal segmental glomerulosclerosis and 5.9 % had glomerulonephritis. Initial treatment modalities in patients who commenced renal replacement therapy (RRT) consisted of peritoneal dialysis, hemodialysis, and preemptive transplantation (Tx) in 61.7, 16.0, and 22.3 %, respectively. The Japanese RRT mortality rate was 18.2 deaths per 1000 person-years of observation.

On behalf of the Japanese Society for Pediatric Nephrology.

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Conclusion The incidence of ESRD is lower in Japanese children than in children of other high-income countries. Since 1998, notably, there has been a marked increase in pre-emptive Tx as an initial treatment modality for Japanese children with ESRD.

Keywords End-stage renal disease · Children · Epidemiology · Renal replacement therapy · Japan

Introduction

End-stage renal disease (ESRD) in children is considered a rare, but serious condition [1]. Information on the epidemiology, demographics, treatment modalities, and mortality of pediatric patients with ESRD are essential for a better understanding of this disease and for improving patient care [2]. This information is also useful for patients, their families, physicians, and other healthcare providers. The epidemiology and demographics of pediatric ESRD have been analyzed in the USA [3, 4], Europe [5–8], and Australia and New Zealand [9, 10]. However, limited information is available on pediatric ESRD patients in other areas of the world.

The Japanese Society for Pediatric Nephrology (JSPN) reported epidemiological and demographic data on Japanese children with ESRD in 1998 [11]. Since then, however, there has been no nationwide survey on Japanese children with ESRD. International comparisons of the epidemiological and demographic characteristics of pediatric ESRD patients may improve outcomes [12, 13]. Therefore, the JSPN ESRD Survey Committee, in collaboration with the Japanese Society for Dialysis Therapy (JSDT) and the Japanese Society for Clinical Renal Transplantation (JSCRT), conducted a nationwide survey of Japanese children with ESRD in 2012. This report describes the basic epidemiological and demographic characteristics of Japanese children aged less than 20 years with ESRD over the period 2006–2011.

Patients and methods

Data collection

The JSPN conducted a cross-sectional nationwide survey in 2012, in collaboration with the JSDT and the JSCRT.

ESRD patients were defined as those with irreversible kidney function disorders requiring renal replacement therapy (RRT) to sustain life. This survey evaluated Japanese patients aged less than 20 years who were newly diagnosed with ESRD between January 1, 2006, and December 31, 2011, and who were followed up until December 31, 2011. Individual patient data included date of birth, gender, primary renal disease, date of starting RRT, treatment modality at the start of RRT, and important events such as death.

Questionnaires were collected in two steps. The first questionnaires, asking about patients newly diagnosed with ESRD, were sent to institutions at which members of the JSPN, JSDT, and JSCRT practiced, as well as to children's hospitals, and pediatric and nephrology departments of medical schools throughout Japan. The second questionnaires, which asked for data about individual patients, were sent to the institutions that reported having new pediatric ESRD patients.

This survey was in accordance with the ethical principles in the 1964 Declaration of Helsinki, and with the ethical guidelines for epidemiological studies issued by the Ministry of Health, Labour and Welfare, Japan. This survey was also approved by the central ethics board of Tokyo Women's Medical University (approval number; 2353) before study commencement.

Data analysis

The incidence of ESRD was defined as the number of new patients with ESRD per year, over the period 2006–2011, and was expressed as number per million age-related population (pmarp), with pmarp calculated using age-, sex-, and year-specific census data obtained from the Japanese data base [14].

Patient survival was analyzed by the Kaplan–Meier life table method. Mortality rates (deaths per 1000 person-years of observation) were also calculated. Data were analyzed using SAS system version 9 (SAS Institute, Cary, NC, USA).

Results

Patients with ESRD

The first questionnaires were sent to a total of 773 institutions, with 770 (99.6 %) responding; of these, 146 institutions had new pediatric ESRD patients during the period 2006–2011. The second questionnaires were therefore sent to these 146 institutions, with 136 (93.2 %) responding. These institutions reported a total of 540 new pediatric ESRD patients, including 322 male patients, 216 female patients and two patients not specified.

Incidence of ESRD

The per-year incidence of ESRD in the years 2006–2011 was 3.5, 3.9, 3.6, 4.7, 4.1, and 4.1 pmarp, respectively.