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Integrated View of the Human Chromosome X-centric Proteome Project

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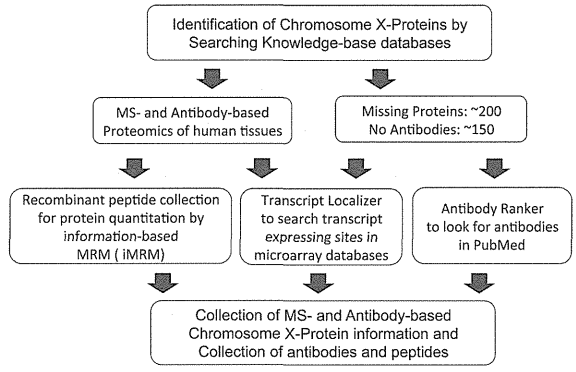
ABSTRACT: This article introduces how the human chromosome X-centric proteome project is carried out by the Japan Chromosome X Project Consortium. The inactivation of one of two chromosomes in female mammals and accumulation of genes related to neural/immune systems/tumor/testis are characteristic of chromosome X. In this Chromosome X Project, information on proteins translated from genes on chromosome X is collected by both mass spectrometry- and antibody-based proteomics. Information on the following resources is also provided: antibodies to proteins translated and full-length cDNAs transcripted from the chromosome X genes for recombinant proteins. The consortium aims to provide the following tools to search useful antibodies in the literature (Antibody Ranker), to find gene expression sites in microarray databases (Transcript Localizer) and to do advanced MRM analysis (information-based MRM).

KEYWORDS: *chromosome X, mass spectrometry, antibody, MRM*



INTRODUCTION

Chromosome X is one of the two sex-determining chromosomes (the other is the Y chromosome) in many animal species, including mammals, and is found in both males and females. This chromosome has the following characteristics, which are not found in other chromosomes: presence of sex-determining genes such as androgen receptor gene, inactivation of one of two chromosomes (X-inactivation), a large number of genes associated with human hereditary diseases, and significant accumulation of genes for networks in neuronal, immune and tumor-related systems.

The Japanese Proteomics Society (JHUPO) was chosen to participate in the Chromosome-centric Human Proteome Project and was asked to be in charge of chromosome X. The Japan Chromosome X Project Consortium (JCXPC) was organized to complete the project by collecting information and resources of all proteins translated from genes located on chromosome X (chromosome X-proteins). Knowledge of the human chromosome X-proteins and the current activities and aims of the chromosome X project are briefly described.

KNOWLEDGE OF HUMAN CHROMOSOME X

1. Characteristics

The human chromosome X spans about 153 million base pairs (4.94%) out of 3100 million base pairs of total human DNA length. The number of genes on chromosome X is presumed to be 888 genes (4.37%) out of total human genes of 20300.[1] The other sex-determining chromosome, Y chromosome, has only 68 genes and one of these genes, named SRY (Sex-determining

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Table 1. Summary of Genes and Proteins on Human Chromosome X

identification level	database	identified/total	%	URL
Transcript	neXtProt	823/874	94.2%	http://www.nextprot.org
neXtProt	neXtProt	615/874	70.4%	http://www.proteinatlas.org/
	Peptide Atlas	396/874	45.3%	http://www.peptideatlas.org
	SRM Atlas	~19000/ ~20300	93.6%[a]	http://www.srmatlas.org
	GPM DB	657/862	76.2%	http://www.thegpm.org
Protein by Antibody	HPA	495/841	55.7%	http://www.proteinatlas.org
	Antibodypedia	722/874	82.6%	http://www.antibodypedia.com
Disorders associated[b]	neXtProt	195/874	22.3%	
	OMIM Gene Map	305/874	34.9%	http://omim.org/geneMap
	Genetics Home Reference	107/874	12.2%	http://ghr.nlm.nih.gov/chromosome/X/show/Conditions

[a]Estimated based on all genes. [b]Including Dominant X-linked diseases (related gene): Vitamin D resistant rickets (X-linked hypophosphatemia, PHEX), Rett syndrome (MECP2), Fragile X syndrome (FMR1), Alport syndrome (COL4A5), etc. X-linked recessive inheritance: Color blindness (OPN1MW, OPN1LW), Hemophilia (F8, F9), Duchenne muscular dystrophy (DMD), X-linked agammaglobulinemia (BTK), Fabry disease (GLA), etc.

Region on the Y chromosome), determines male by inducing and developing the testis to produce a male hormone, androgen. On the chromosome X only a few of the 888 genes directly play a role in sex determination. One is the gene encoding androgen receptor on the chromosome X, indicating the importance of chromosome X in males and suggesting a cross-communication between chromosomes X and Y and also the significance of the androgen receptor in females.

Besides the sex-determining genes, genes in the neural system are uniquely clustered on chromosome X: NLGN3 (Neuroligin-3), NLGN4X (Neuroligin-4, X-linked), OPHN1 (Oligophrenin-1), PAK3 (Serine/threonine-protein kinase), FMR1 (fragile X mental retardation 1), MAG (myelin associated glycoprotein) and others. Proteins translated from these genes are essential for interaction or communication of neurons and are presumed to relate to the intelligence.[2] The important role of the X chromosome in brain function is also evident from the prevalence of X-linked forms of mental retardation.

The accumulation of immune system-related genes to chromosome X also attracts attention. CD40L (CD40 ligand), IL2RG (Cytokine receptor common subunit gamma), BTK (Tyrosine-protein kinase), F8 (Coagulation factor VIII), and F9 (Coagulation factor IX) are example of chromosome X genes that are involved in the immune system and coagulation system.[3]

The inactivation of chromosome X is a process by which one of the two copies of chromosome X in females is inactivated. The inactive X chromosome is transcriptionally silenced to form an inactive structure called heterochromatin. The choice of which X chromosome is inactivated is randomly occurring in each cell in mammals. The X-inactivation center on the X chromosome, which is essential to cause X-inactivation, contains four nontranslated RNA genes, Xist, Tsix, Jpx and Ftx, which are involved in X-inactivation.[4,5]

2. Human Chromosome X-Proteins Identified by Mass Spectrometry (MS)

Information of genes on chromosome X and the proteins encoded by the genes has been collected in several databases (Table 1). In the neXtProt database (http://www.nextprot.org), 874 genes are presumed on chromosome X.[1] Among them, 823 (94.2%) genes have been identified at the transcript level and 615 (70.4%) genes have been demonstrated at the protein level by proteomics. In the other proteome databases, Peptide Atlas and GPM DB (Global Proteome Machine database), 45.3 and 75.2%, respectively, of the genes on chromosome X are identified as proteins. However, these data indicate that more than 200 genes on chromosome X are still unclear whether they translate proteins or not. These unclear proteins are further confirmed by MS and immunohistochemistry using antibodies in this project.

3. Proteomes Identified by Antibody-based Methods

Collection and validation of antibodies against human proteins are progressing by Human Protein Atlas project.[6] By using antibodies, localization of 495 (56.6%) chromosome X-proteins has been examined at cellular and subcellular levels in human body.

The Antibodypedia is a Web site providing datasheets of antibodies against human proteins from antibody providers (http://www.antibodypedia.com). In this collection, datasheets of antibodies against 722 (82.6%) chromosome X-proteins are currently shown although these antibodies have not always been well-characterized in the specificity or reactivity to the proteins for immunolocalization. The Chromosome X Project Consortium members will collect significant evidence of the presence or localization of the chromosome X-proteins from the literature or from their own research.

4. Diseases Associated with Chromosome X

A large number of genes (195 in the neXtProt database) in chromosome X have been demonstrated to associate with genetic disorders and hereditary diseases in humans (Table 1). One of the reasons is only one copy of chromosome X is active both in males (XY) and females (XX) (X-inactivation), resulting in prevalence of X-linked hereditary diseases.

It is estimated that about 10% of the genes (99 genes) encoded by the X chromosome are associated with a family of "CT antigen (cancer-testis antigen)" genes, which encode for markers found in both cancer cells as well as in the human testis (MAGE, GAGE, SSX, SPANX or other CT gene families).[7]

THE JAPAN CHROMOSOME X PROJECT

1. Selection of Tissues and Organs

Since preference in expression of chromosome X genes in neural and immune systems and the tissues (neural and immune systems or cancers and testis) has been demonstrated as described above, it is presumed that expression of chromosome X-proteins is also different among organs or tissues. Therefore, expression of chromosome X-proteins were searched in the kidney, brain, ovary and testis in the Human

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Protein Atlas. As shown in Table 2, there was no significant preference in the expression among the organs. Therefore, the

Table 2. Identification of Chromosome X-Proteins in the Human Protein Atlas[a]

		immunohistochemistry	
	antibodies used	strong (%)	weak (%)
Placenta	627	148 (23.6)	490 (78.1)
Kidney	900	149 (16.6)	654 (72.7)
Ovary	1180	160 (13.6)	825 (69.9)
Testis	1032	224 (21.7)	821 (79.6)
Brain	1112	159 (14.3)	776 (69.8)

[a]Placenta, kidney, ovary, testis and brain tissues were examined by immunohistochemistry using antibodies in the Human Protein Atlas (http:www.proteinatlas.org/). Numbers of antibodies used, stained the tissues strongly or more than weakly are shown (%).

Japan chromosome X project consortium preliminarily chose kidney, ovary, and breast as target sample tissues to look for chromosome X-proteins, which had not been well-identified yet because these organs had not been analyzed by other chromosome projects and our project members had already analyzed the proteomes of these organs more or less.

2. Collection of Protein Existence by MS

With informed consent, human kidney, ovary, breast tissues were obtained from patients when these organs or tissues were surgically removed for treatment of cancers. Kidneys were separated into cortex, medulla and glomerulus.[8] More fine structured (proximal tubule, distal tubule, collecting duct, and others) kidney sections were microdissected from kidney sections by laser microdissection system for deeper and more comprehensive MS analysis of kidney nephron parts. Other organs are also considered for such in depth MS analysis.

Members of the Japan Chromosome X Project are interested in MS analysis of cancers[9−12] and biofluids[13,14] for biomarker discovery and understanding of pathophysiology of cancers. Other members are also focusing on analysis of protein modification such as phosphorylation or glycosylation and collect MS evidence of post-translational modifications of chromosome X-proteins in the target organs and others.[15]

Another approach to find possible tissue or organ sites was carried out to develop a search engine ("Transcript Localizer") to look at human microarray databases and to pick up sites where missing or unclear chromosome X genes are detected.

3. Collection of Protein Localization by Antibodies

Cellular localization of proteins, which would first be identified by MS in the target organs, was secondarily searched in the Human Protein Atlas database and the immunohistochemistry images were retrieved to combine to the data obtained by MS-based proteomics. A prototype of the human kidney proteome database has been opened to the public at the Web site of the HUPO Human Kidney and Urine Proteome Project (HKUPP) Initiative (www.hkupp.org/). The members of the chromosome X project also examined localization of MS-identified proteins in the target organs by immunohistochemistry to confirm the Human Protein Atlas data and the MS identification results. Our consortium will collect information on antibodies to the proteins, which are not provided by the Human Protein Atlas project, by searching in the Antibodypedia.

We are also developing an antibody search engine tool that looks for antibodies in open free access articles in the PubMed database and picks up antibodies to human proteins and collects the following information: name of companies providing antibodies and images obtained by the antibodies. Several different antibodies to one human protein were used in the past studies and the search engine collects information of all of these antibodies and will demonstrate antibody information in a rank order of number of articles in which the same antibody was used. This informs us which antibody is mostly used for a human protein in a community of scientists. Therefore, the tool was named "Antibody Ranker". We believe this tool provides valuable information for researchers who are looking for antibodies for human proteins. Efficiency of the Antibody Ranker is also validated by selecting antibodies for immunohistochemistry in the chromosome X project.

4. Resources and Tools Developed from the Chromosome X Project

The Human Gene and Protein Database (HGPD; http://www.HGPD.jp/) launched in 2008 is a unique resource storing 43249 human Gateway entry clones constructed from the open reading frames (ORFs) of full-length human cDNA, which is the largest in the world.[16] Since the set of these clones are used for recombinant human protein synthesis by the wheat germ cell-free protein synthesis system, this resource is named the Human Proteome Expression Resource (HuPEX).[17] The recombinant protein resource has covered more than 85% of human proteins encoded by 20300 genes.

All synthesized proteins (approx. 18000) have been intensively analyzed by MS after trypsinization and MS/MS information of individual peptides from the proteins has been collected as a database for selection of peptides and MRM (multiple reaction monitoring) transitions. This provides us information to select peptides and MS/MS transitions for MRM (information-based MRM, iMRM) and also resources of reference peptides for quantitation of proteins in the target tissues.

CONCLUSION

Current status and future plans of the Japan human chromosome X project are summarized in Figure 1 .. Collaboration and cooperation with other chromosome

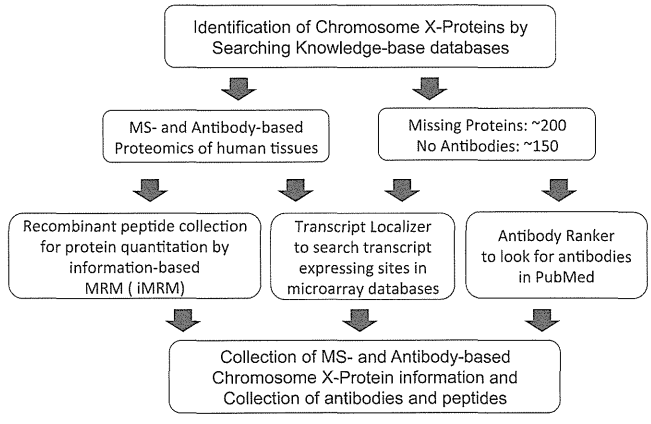

Figure 1. Workflow of Japan Chromosome X Project. Shown here is a strategy from basic collection of knowledge-base proteomics data to final completion of chromosome X proteome data and resource collection done by Japan Chromosome X Project Consortium.

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projects in the Chromosome-centric Human Proteome Project, especially chromosome Y and with other Biology/Disease Human Proteome projects, need to be facilitated to complete the Human Proteome Project.

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Notes

The authors declare no competing financial interest.

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ORIGINAL ARTICLE

Renal disease in the elderly and the very elderly Japanese: analysis of the Japan Renal Biopsy Registry (J-RBR)

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Abstract

Background and objectives Data regarding renal disease in the elderly (age ≥65 years old) and very elderly (age ≥80 years old) Japanese are extremely limited. The aim of this study was to examine the causes of renal disease and their clinical presentations in elderly patients who underwent renal biopsy.

Design, setting, participants, and measurements From July 2007 to November 2011, all of the elderly native renal biopsy patients who had been registered in the Japan Renal Biopsy Registry (J-RBR; 2802 including 1596 males and 1206 females) were identified. Their data were compared with a control group of 7416 patients who ranged in age from 20 to 64 years old and were registered on the J-RBR

On behalf of the Committee for the Standardization of Renal Pathological Diagnosis and for Renal Biopsy and Disease Registry of the Japanese Society of Nephrology, and the Progressive Renal Disease Research of the Ministry of Health, Labour and Welfare of Japan.

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over the same period. In addition, the clinical and pathological classifications of 276 very elderly patients were also analyzed.

Results The indications for biopsy were nephrotic syndrome (NS) in 36.2 and 50.7 % of the elderly and the very elderly patients, chronic nephritic syndrome in 31.8 and 17.4 %, and acute kidney injury including rapidly progressive glomerulonephritis in 18.6 and 22.5 %, respectively. Primary glomerular disease was the most frequent diagnosis, followed by MPO-ANCA-positive nephritis, IgA nephropathy (IgAN), and diabetic nephropathy. In primary GN including IgAN, membranous nephropathy (MN) was the most frequent histological type, followed by IgAN and minor glomerular abnormalities. A comparison with the control group showed that MN, MPO-ANCA-positive nephritis, and amyloid nephropathy were more common in the elderly ($P < 0.001$), and IgAN was less common ($P < 0.001$). As for nephrotic syndrome in the elderly, MN was the most common histological type, followed by minimal change NS, diabetic nephropathy, amyloid nephropathy, and focal segmental glomerulosclerosis. There was a significant discrepancy between the urinary protein/creatinine ratio and daily proteinuria after the 7th decade of life.

Conclusions Renal biopsy is a valuable diagnostic tool, even in elderly and very elderly Japanese patients. In the future, modified clinical guidelines for elderly renal disease should be developed.

Keywords Elderly · Very elderly · Japanese · Renal biopsy · Registry · Nephrotic syndrome · IgA nephropathy · Rapidly progressive glomerulonephritis · Proteinuria

Introduction

In Japan, the elderly population; i.e., those aged 65 and over, accounted for 25.8 % of the total population in October 2010, and this will increase to 30.5 % by 2025 [1]. As life expectancy increases, more elderly patients with acute and chronic renal diseases are surviving longer. In particular, the progressive decline in GFR that occurs with age; the decline in GFR brought about by cardiovascular and other systemic diseases, such as anti-neutrophil cytoplasmic antibody (ANCA)-positive vasculitis; and reductions in GFR due to the nephrotoxic effects of medical/surgical treatment are expected to contribute to an increased incidence of renal disease in the elderly population. Several studies involving limited numbers of elderly Japanese patients have suggested that renal biopsy can provide significant diagnostic and prognostic information

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[2–5]. In addition, only three studies that included a subset analysis of patients aged over 75–80 years old have been reported from Europe or the USA, and these involved limited numbers of patients [6–8].

Until recently, there were no web-based, nationwide, or prospective registry systems for renal biopsies in Japan. Thus, in 2007, the Committee for the Standardization of Renal Pathological Diagnosis and the Working Group for the Renal Biopsy Database of the Japanese Society of Nephrology established the first nationwide, Web-based, and prospective registry system, the Japan Renal Biopsy Registry (J-RBR), to record pathological, clinical, and laboratory data about the renal biopsies performed in Japan [9].

The aim of this study was to examine the specific causes of renal disease and their respective clinical presentations in a large group of elderly (over 65 years old) patients and very elderly patients (over 80 years old) who had undergone native renal biopsy and to compare the frequencies of their diagnoses with those of a control group of patients who ranged in age from 20 to 64 years.

Materials and methods

J-RBR system and subjects

The researchers of the Committee for the Standardization of Renal Pathological Diagnosis and the Working Group for the Renal Biopsy Database of the Japanese Society of Nephrology set up the J-RBR [9]. This study includes data obtained from 12705 renal-biopsied patients that were prospectively registered in the J-RBR from July 2007 to November 2011.

Patient data including age, gender, laboratory data, clinical category, and pathological diagnosis were electronically recorded at each institution and registered on the J-RBR webpage via the Internet Data and Information Center for Medical Research (INDICE) system, which is part of the University Hospital Medical Information Network (UMIN). The ethics committee of the Japanese Society of Nephrology comprehensively approved the study, and the local committees of the participating centers and their affiliate hospitals individually approved the study. The J-RBR was registered to the Clinical Trial Registry of UMIN (UMIN000000618).

Clinical categories

The clinical categories of glomerular disease were defined as follows: nephrotic syndrome, chronic nephritic syndrome, recurrent or persistent hematuria, acute nephritic syndrome (AGN), and rapidly progressive nephritic syndrome (RPGN), based on the criteria developed by the WHO. The definitions of these five clinical diagnoses were based on their clinical symptoms and glomerular

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Table 1 Frequency of classification of clinical diagnoses in the elderly Japanese (≥65 years old)

	Very elderly (≥80 years old)		Elderly (≥65 years old)		Control (20–64 years old)		*P* value*
Cases	276		2802		7416		
Gender (male:female)	141:135		1596:1206		3795:3621		
Clinical classification	*n*	%	*n*	%	*n*	%	
Nephrotic syndrome	140	50.7	1018	36.3	1359	18.3	<0.001
Chronic nephritic syndrome	48	17.4	870	31.0	4434	59.8	<0.001
Rapidly progressive nephritic syndrome (RPGN)	54	19.6	432	15.4	300	4.0	<0.001
Acute nephritic syndrome (AGN)	2	0.7	40	1.4	122	1.6	NS
Recurrent or persistent hematuria	1	0.4	33	1.2	263	3.5	<0.001
Renal disorder with collagen disease or vasculitis	12	4.3	117	4.2	326	4.4	NS
Renal disorder with metabolic syndrome	4	1.4	69	2.5	160	2.2	NS
Hypertensive nephropathy	1	0.4	42	1.5	108	1.5	NS
Acute kidney injury (AKI)	6	2.2	51	1.8	55	0.7	<0.001
Drug-induced nephropathy	1	0.4	16	0.6	46	0.6	NS
Inherited renal disease	2	0.7	4	0.1	21	0.3	NS
Thrombotic microangiopathy (TMA, HUS/TTP[a])	0	0.0	0	0.0	3	0.0	NS
Others	5	1.8	110	3.9	219	3.0	0.03

NS not significant

* The elderly versus controls

[a] Hemolytic uremic syndrome/thrombotic thrombocytopenic purpura

histopathology, as described in the WHO classification of glomerular diseases [10]. Acute nephritic syndrome was defined as a syndrome characterized by the abrupt onset of hematuria, proteinuria, hypertension, decreased glomerular filtration, and edema. Rapidly progressive nephritic syndrome was defined as the abrupt or insidious onset of hematuria, proteinuria, anemia, and rapidly progressive renal failure. Recurrent or persistent hematuria was defined as the insidious or abrupt onset of gross or microscopic hematuria with little or no proteinuria and no evidence of other features of nephritic syndrome. Chronic nephritic syndrome was defined as slowly developing renal failure accompanied by proteinuria, hematuria, with or without hypertension. Nephrotic syndrome was defined as proteinuria of ≥3.5 g/day and/or 3.5 g/gCr with hypoalbuminemia (serum albumin <3.0 g/dl) and/or hypoproteinemia (total protein <6.0 g/dl) according to the Progressive Renal Diseases Research (2011) criteria [11].

In addition, secondary diseases and tubulointerstitial diseases were categorized as follows: renal disorder with collagen disease or vasculitis, renal disorder with metabolic syndrome; hypertensive nephropathy; acute kidney injury; drug-induced nephropathy; inherited renal disease; thrombotic microangiopathy (TMA); hemolytic uremic syndrome (HUS); thrombotic thrombocytopenic purpura (TTP); and others, which included acute interstitial injuries, chronic interstitial injury, and acute tubular necrosis, as described previously [9].

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Table 2 Frequency of pathological diagnoses as classified by pathogenesis in the elderly Japanese (≥65 years old)

	Very elderly (≥80 years old)		Elderly (≥65 years old)		Control (20–64 years old)		*P* value*
	n	%	*n*	%	*n*	%	
Primary glomerular disease	124	44.9	1259	44.9	5021	60.4	<0.001
Primary glomerulonephritis (except for IgAN)	105	38.0	966	34.5	1666	22.5	<0.001
IgA nephropathy (IgAN)	19	6.9	293	10.5	2815	38.0	<0.001
Secondary and hereditary glomerular diseases	100	36.2	1003	35.8	1766	23.8	<0.001
MPO-ANCA-positive nephritis	31	11.2	313	11.2	164	2.2	<0.001
Diabetic nephropathy	16	5.8	215	7.7	399	5.4	<0.001
Hypertensive nephropathy	14	5.1	173	6.2	304	4.1	<0.001
Amyloid nephropathy	20	7.2	110	3.9	58	0.8	<0.001
Purpura nephritis	4	1.4	56	2.0	151	2.0	NS
Lupus nephritis	4	1.4	44	1.6	461	6.2	<0.001
Infection-related nephropathy	5	1.8	41	1.5	65	0.9	0.012
Anti-glomerular basement membrane antibody-type nephritis	1	0.4	17	0.6	21	0.3	<0.001
PR3-ANCA-positive nephritis	3	1.1	13	0.5	21	0.3	NS
Thrombotic microangiopathy	0	0.0	10	0.4	20	0.3	NS
Dense deposit disease (MPGN type II)	2	0.7	8	0.3	2	0.2	NS
Alport syndrome	0	0.0	2	0.1	27	0.4	NS
Thin basement membrane disease	0	0.0	1	0.0	73	1.0	0.002
Tubulointerstitial diseases	16	5.8	149	5.3	142	1.9	<0.001
Chronic tubulointerstitial lesions	6	2.2	69	2.5	38	0.5	NS
Acute tubulointerstitial lesions	9	3.3	71	2.5	87	1.2	NS
Acute tubular necrosis	1	0.4	9	0.3	17	0.2	NS
Others	36	13.0	391	14.0	126	1.7	NS
Total	276	100	2802	100	7416	100	

NS not significant

* The elderly versus controls

Table 3 Frequency of pathology in the primary glomerular disease of the elderly Japanese (≥65 years old)

	Elderly (≥65 years old)		Control (20–64 years old)		*P* value*
	n	%	*n*	%	
IgA nephropathy (IgAN)	293	23.3	2815	56.1	<0.001
Membranous nephropathy	485	38.5	455	9.1	<0.001
Minor glomerular abnormalities	156	12.4	832	16.6	<0.001
Focal segmental glomerulosclerosis	99	7.9	327	6.5	NS
Membranoproliferative glomerulonephritis (MPGN type I and III)	75	6.0	83	1.7	<0.001
Dense deposit disease (DDD, MPGN type II)	0	0.0	8	0.2	NS
Crescentic glomerulonephritis	30	2.4	26	0.5	NS
Non-IgA mesangial proliferative glomerulonephritis	69	5.5	365	7.3	<0.001
Endocapillary proliferative glomerulonephritis	15	1.2	34	0.7	NS
Other/unclassifiable	37	2.9	76	1.5	NS
Total	1259	100	5021	100	

NS not significant

* The elderly versus controls

Table 4 Frequency of pathogenesis classified by clinical classification in the elderly Japanese (≥65 years old)

Classification	Nephrotic syndrome[a]		Chronic nephritic syndrome		Rapidly progressive nephritic syndrome		Acute nephritic syndrome		Recurrent or persistent hematuria		Subtotal
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	
Primary glomerulonephritis (except for IgAN)	613	59.5	184	29.0	29	9.3	13	46.4	3	14.3	842
IgA nephropathy (IgAN)	40	3.9	154	24.3	11	3.5	2	7.1	4	19.0	211
MPO-ANCA-positive nephritis	19	1.8	15	2.4	170	54.7					204
Daibetic nephropathy	100	9.7	33	5.2	1	0.3			1	4.8	135
Hypertensive nephropathy	17	1.6	69	10.9	7	2.3	1	3.6	2	9.5	96
Amyloid nephropathy	79	7.7	9	1.4	3	1.0					91
Infection-related nephropathy	14	1.4	8	1.3	8	2.6	5	17.9	1	4.8	36
Purpura nephritis	12	1.2	12	1.9	5	1.6			2	9.5	31
Lupus nephritis	13	1.3	8	1.3	3	1.0	1	3.6			25
Anti-glomerular basement membrane antibody-type nephritis					10	3.2					10
PR3-ANCA-positive nephritis	1	0.1			7	2.3					8
Thrombotic microangiopathy	1	0.1			1	0.3					2
Alport syndrome			1	0.2							1
Thin basement membrane disease									1	4.8	1
Others/unclassifiable	122	11.8	141	22.2	56	18.0	6	21.4	7	33.3	332
											0
Subtotal	1031	100	634	100	311	100	28	100	21	100	2025

[a] Including cases with other classifications who satisfied the 2011 criteria of nephrotic syndrome in Japan

Table 5 Frequency of histopathology classified by clinical classification in the elderly Japanese (≥65 years old)

Classification	Nephrotic syndrome[a]		Chronic nephritic syndrome		Rapidly progressive nephritic syndrome		Acute nephritic syndrome		Recurrent or persistent hematuria		Subtotal
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	
Membranous nephropathy	383	37.1	102	16.1	2	0.6	2	7.1	1	4.8	490
Mesangial proliferative glomerulonephritis	74	7.2	236	37.2	21	6.8	4	14.3	7	33.3	342
Crescentic glomerulonephritis	42	4.1	19	3.0	207	66.6	3	10.7			271
Minor glomerular abnormalities	142	13.8	18	2.8	1	0.3			2	9.5	163
Nephrosclerosis	38	3.7	85	13.4	7	2.3	1	3.6	2	9.5	133
Focal segmental glomerulosclerosis	71	6.9	31	4.9	4	1.3	1	3.6			107
Membranoproliferative glomerulonephritis (MPGN type I and III)	67	6.5	27	4.3	4	1.3	3	10.7			101
Endocapillary proliferative glomerulonephritis	17	1.6	2	0.3	9	2.9	9	32.1	2	9.5	39
Dense deposit disease (DDD, MPGN type II)	4	0.4	2	0.3							6
Sclerotic glomerulonephritis	22	2.1	19	3.0	6	1.9			2	9.5	49
Acute interstitial nephritis	3	0.3	4	0.6	14	4.5	2	7.1	1	4.8	24
Chronic interstitial nephritis	1	0.1	13	2.1	6	1.9	1	3.6	1	4.8	22
Acute tubular necrosis	1	0.1									1
Other/unclassifiable	166	16.1	76	12.0	30	9.6	2	7.1	3	14.3	277
Subtotal	1031	100	634	100	311	100	28	100	21	100	2025

[a] Including cases with other classifications who satisfied the 2011 criteria of nephrotic syndrome in Japan

Table 6 Pathological diagnoses of nephrotic syndrome in the elderly Japanese (≥65 years old)

	Elderly (≥65 years old)		Control (20–64 years old)		*P* value*
	n	%	*n*	%	
Primary nephrotic syndrome including IgAN	718	61.9	965	60.7	
Membranous nephropathy	365	31.5	284	17.9	<0.001
Minimal change nephrotic syndrome	146	12.6	403	25.3	<0.001
Focal segmental glomerulosclerosis	68	5.9	110	6.9	NS
Membranoproliferative glomerulonephritis (type I/III)	51	4.4	28	1.8	<0.001
Mesangial proliferative glomerulonephritis except for IgAN	17	1.5	12	0.8	NS
Crescentic glomerulonephritis	10	0.9	5	0.3	NS
Endocapillary proliferative glomerulonephritis	8	0.7	9	0.6	NS
Sclerotic glomerulonephritis	1	0.1	2	0.1	NS
IgA nephropathy (IgAN)	48	4.1	106	6.7	0.006
Others	4	0.3	6	0.4	NS
Secondary nephrotic syndrome	442	38.1	626	39.3	
Diabetic nephropathy	115	9.9	184	11.6	NS
Amyloid nephropathy	88	7.6	37	2.3	<0.001
Lupus nephritis	18	1.6	160	10.1	<0.001
Infection-related nephropathy	17	1.5	21	1.3	NS
Nephrosclerosis	17	1.5	9	0.6	0.016
Purpura nephritis	16	1.4	21	1.3	NS
MPO-ANCA-positive nephritis	19	1.6	14	0.9	NS
PR3-ANCA-positive nephritis	1	0.1	1	0.1	NS
Anti-glomerular basement membrane antibody-type nephritis	0	0.0	3	0.2	NS
Alport syndrome	1	0.1	6	0.4	NS
Thrombotic microangiopathy	1	0.1	3	0.2	NS
Others	149	12.8	167	10.5	NS
Total	1160	100	1591	100	

NS not significant

* The elderly versus controls

Pathological diagnoses

The patients' renal histological diagnoses were classified according to their pathogenesis (A) or histopathology (B) as follows: (A) primary glomerular disease (except IgA nephropathy, IgAN), IgAN, purpura nephritis, lupus nephritis, myeloperoxidase(MPO)-ANCA-positive nephritis, protein 3 (PR3)-ANCA-positive nephritis, anti-glomerular basement membrane antibody nephritis, hypertensive nephrosclerosis, thrombotic microangiopathy, diabetic nephropathy, amyloid nephropathy, Alport syndrome, thin basement membrane disease, infection-related nephropathy, kidney transplantation, and others; (B) minor glomerular abnormalities, focal and segmental glomerulosclerosis (FSGS), membranous nephropathy (MN), mesangial proliferative glomerulonephritis, endocapillary proliferative glomerulonephritis, membranoproliferative glomerulonephritis (MPGN) (types I and III), dense deposit disease (DDD, MPGN type II), crescentic and necrotizing glomerulonephritis, sclerosing glomerulonephritis, nephrosclerosis, acute interstitial nephritis, chronic interstitial nephritis, acute tubular necrosis, kidney transplantation, and others. IgAN (Berger's disease) is separated from primary glomerular disease on the basis of basic glomerular alterations in the WHO classification of glomerular diseases [10].

Clinical data, including urinalysis results; daily proteinuria values; and serum creatinine, total protein, albumin, and total cholesterol values, were also recorded.

Statistical analyses

Continuous variables are reported as mean values (standard deviation, SD). Statistical analyses were performed using SPSS version 18.0 (SPSS, Tokyo, Japan). Comparisons of

Table 7 Pathological diagnoses of nephrotic syndrome in the very elderly Japanese (≥80 years old)

	n	%
Primary nephrotic syndrome (male:female)	95 (37:58)	59.4
Membranous nephropathy	45	28.1
Minimal change nephrotic syndrome	19	11.9
Focal segmental glomerulosclerosis	12	7.5
Membranoproliferative glomerulonephritis (type I/III)	4	2.5
Mesangial proliferative glomerulonephritis except for IgA nephropathy	4	2.5
Crescentic glomerulonephritis	2	1.3
Endocapillary proliferative glomerulonephritis	2	1.3
IgA nephropathy	7	4.4
Secondary nephrotic syndrome except for IgA nephropathy (male:female)	65 (33:32)	40.6
Diabetic nephropathy	10	6.3
Amyloid nephropathy	19	11.9
Lupus nephritis	1	0.6
Infection-related nephropathy	3	1.9
Nephrosclerosis	4	2.5
Purpura nephritis	0	0.0
MPO-ANCA-positive nephritis	3	1.9
Others	25	15.6
Total cases (male:female)	160 (70:90)	100

categorical variables among groups of different indications or diagnoses were performed using Fischer's exact test. Continuous variables were compared using the Student's *t* test for parametric data and Wilcoxon's signed rank test or the Kruskal-Wallis test for non-parametric data. *P* values of <0.05 (obtained by two-tailed testing) were considered to indicate statistical significance.

Results

The elderly and very elderly patients in the J-RBR (2007–2011)

At the end of November 2011, 2802 patients who were more than 65 years old (27.4 %) and 276 who were older than 80 (2.7 %) were extracted from the 10218 adult (over 20 years old) patients registered in the J-RBR. We analyzed the frequency of each clinical diagnosis (indication for renal biopsy), pathogenesis, and histopathological diagnosis in the elderly population and controls.

The indications for biopsy were nephrotic syndrome in 36.2 and 50.7 % of the elderly and very elderly patients; chronic nephritic syndrome in 31.0 and 17.4 %; and acute kidney injury (AKI) including RPGN, AGN, and ATN in 18.6 and 22.5 %, respectively (Table 1).

Table 8 Frequency of pathogenesis in RPGN of the elderly Japanese (≥65 years old)

	Total		Elderly (≥65 years old)		Control (20–64 years old)		*P* value*
Cases	732		432		300		
Gender (male:female)	(404:328)		(242:190)		(162:138)		
	n	%	*n*	%	*n*	%	
Type I: Anti-glomerular basement membrane antibody-type nephritis	31	4.2	13	3.0	18	6.0	0.0646
Type II: Immune-complex (IC) type	195	26.6	91	21.1	104	34.7	0.0026
Primary glomerulonephritis (except for IgAGN)	60	8.2	36	8.3	24	8.0	NS
IgA nephropathy (IgAGN)	57	7.8	20	4.6	37	12.3	0.0007
Secondary IC diseases	35	4.8	16	3.7	19	6.3	NS
Purpura nephritis	16	2.2	8	1.9	8	2.7	NS
Lupus nephritis	14	1.9	3	0.7	11	3.7	0.0058
Infection-related nephropathy	13	1.8	8	1.9	5	1.7	NS
Type III: Pauci immune type	502	68.6	329	76.2	173	57.7	0.0235
MPO-ANCA-positive nephritis	350	47.8	245	56.7	105	35.0	0.0005
PR3-ANCA-positive nephritis	15	2.0	9	2.1	6	2.0	NS
Systemic vasculitis	137	18.7	75	17.4	62	20.7	NS
Thrombotic microangiopathy	5	0.7	1	0.2	4	1.3	NS
Others	33	4.5	14	3.2	19	6.3	NS

NS not significant

* The elderly versus controls

Table 9 Case profiles and clinical diagnoses of IgA nephropathy in the elderly (≥65 years old)

Cases/gender (male:female):	Total		Elderly (≥65 years old)		Control (20–64 years old)		*P* value
	3109 (1559:1550)		293 (189:104)		2816 (1370:1446)		
	n	%	*n*	%	*n*	%	
Male gender	1559	50.1	189	63.4	1370	48.7	0.005
Proteinuria (≥1+)	2529	81.3	252	86.0	2277	80.9	NS
Hematuria (≥1+)	2686	86.4	251	85.7	2435	86.5	NS
CKD stage (1–3a vs. 3b–5)*							0.0117*
Stage G1	1074	35.0	23	7.9	1051	37.8	
Stage G2	950	31.0	74	25.4	876	31.5	
Stage G3a	488	15.9	64	22.0	424	15.3	
Stage G3b	337	11.0	63	21.6	274	9.9	
Stage G4	172	5.6	56	19.2	116	4.2	
Stage G5	48	1.6	11	3.8	37	1.3	
Clinical diagnosis							
Chronic nephritic syndrome	2765	88.9	228	77.8	2537	90.1	NS
Recurrent or persistent hematuria	140	4.5	8	2.7	132	4.7	NS
Nephrotic syndrome	97	3.1	29	9.9	68	2.4	<0.001
Rapidly progressive nephritic syndrome	57	1.8	20	6.8	37	1.3	<0.001
Acute nephritic syndrome	21	0.7	3	1.0	18	0.6	NS
Acute renal failure	6	0.2	2	0.7	4	0.1	NS
Hypertensive nephropathy	3	0.1			3	0.1	NS
Renal disorder with metabolic disease	1	0.0			1	0.0	NS
Others	19	0.6	3	1.0	16	0.6	NS

Table 10 Clinical and laboratory parameters of IgA nephropathy in the elderly (≥65 years old)

	Elderly (≥65 years old)			Control (20–64 years old)			*P* value*
	n	Mean	SD	*n*	Mean	SD	
Daily proteinuria (g/day)	198	1.7	2	2016	1.1	1.4	<0.001
Urinary protein/creatinine ratio (g/gCr)	174	2.6	3.3	1868	1.3	1.7	<0.001
Serum creatinine (mg/dl)	292	1.40	1.10	2808	1.00	0.80	<0.001
Estimated GFR (ml/min/1.73 m^2)	291	51.4	25.3	2778	81.1	34.9	<0.001
Serum total protein (g/dl)	288	6.7	0.9	2794	6.9	0.7	<0.001
Serum albumin (g/dl)	288	3.5	0.7	2776	4	0.5	<0.001
Serum total cholesterol (mg/dl)	280	208	50	2710	204	47	NS
Systolic blood pressure (mmHg)	225	139	19	2323	124	17	<0.001
Diastolic blood pressure (mmHg)	224	78	12	2323	76	19	NS
Mean blood pressure (mmHg)	224	98	12	2323	92	16	<0.001
HbA1c (%)	169	5.5	0.6	1360	5.2	0.6	<0.001

NS not significant

* The elderly vs. controls

As for the pathogenesis of renal biopsy in the elderly and very elderly patients, primary glomerular disease was the most frequent diagnosis ($n = 966$, 34.5 %; $n = 105$, 38.0 %), followed by MPO-ANCA positive nephritis ($n = 313$, 11.2 %; $n = 31$, 11.2 %), IgA nephropathy ($n = 293$, 10.5 %; $n = 19$, 6.9 %), diabetic nephropathy ($n = 215$, 7.7 %; $n = 16$, 5.8 %), hypertensive nephropathy ($n = 173$, 6.2 %; $n = 14$, 5.1 %), and amyloid nephropathy ($n = 110$, 3.9 %; $n = 20$, 7.2 %) (Table 2). Some rare glomerular and tubulointerstitial diseases were recorded in the "others" category, such as immunotactoid glomerulopathy in 3 cases, fibrillary glomerulopathy in 1 case, lipoprotein glomerulopathy in 1 case, glomerulopathy with lecithin-

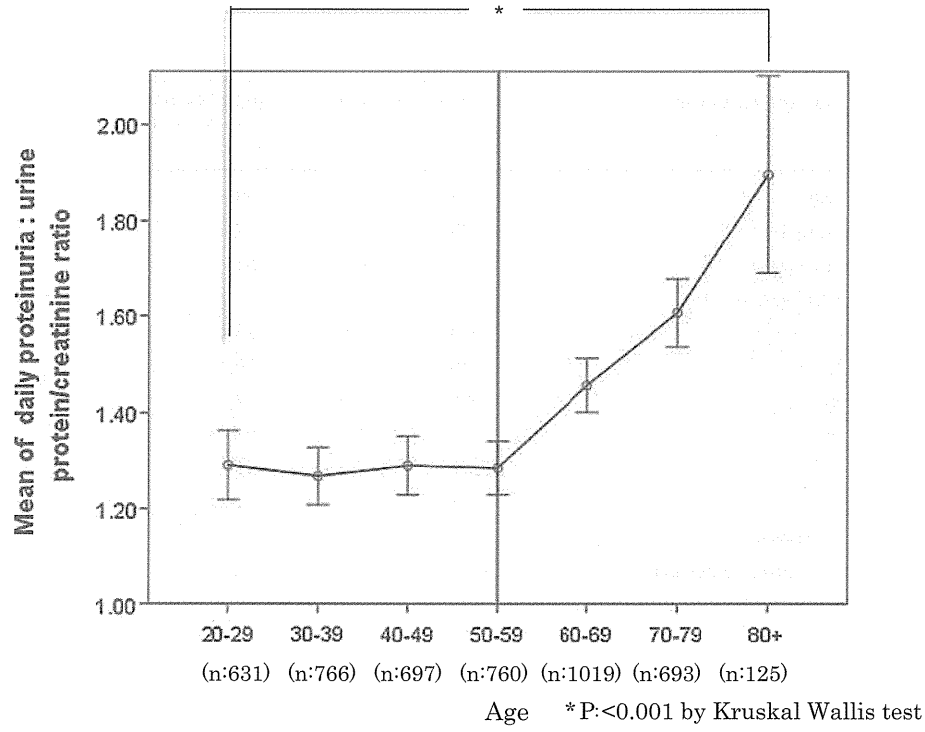

Fig. 1 Discrepancy between daily proteinuria and the urinary protein/creatinine ratio in the elderly. There was a discrepancy between the urinary protein/creatinine ratio (g/gCr) [*A*] and daily proteinuria (g/day) [*B*] after the 7th decade of life. The mean ratio of [*A*]/[*B*] was around 1.26–1.29 from the 3rd to 6th decade; however, it increased significantly to 1.46 in the 7th decade, 1.61 in the 8th decade, and 1.90 in the 9th decade or later (Kruskal–Wallis test, $P < 0.001$)

Table 11 Clinical syndromes of renal biopsy in the elderly

Author	Shin	Prakash	Rivera		Ferro	Brown	Nair	Pincon
Country	South Korea	India	Spain		Italy	Ireland	USA	France
Reported year	2001	2003	2004		2006	2012	2004	2010
Study period	1980–1994	1998–2002	1994–2001		1991–2000	1994–2009	2001–2003	2000–2007
Type of registry	Single center	Single center	Nation wide		Single center	Nation wide	Multicenter	Single center
Total cases	1908	ND	9378		392	1372	7257	ND
Elderly cases[a]	117	65	2173		150	236	413	150
Age	>60 years old	≥60 years old	≥65 years old		>65 years old	≥65 years old	66–79 years old	≥70 years old
Gender (*n* or male:female ratio)	Both (76:41)	Both (56:9)	Male (1305)	Female (868)	Both (ND)	Both (150:86)	Both (1.5:1)	Both (78:72)
Nephrotic syndrome	64.1 %	40.0 %	36.6 %	36.2 %	42.0 %	25.0 %	33.0 %	30.0 %
Rapidly progressive nephritic syndrome (RPGN)	6.8 %	4.0 %	ND	ND	13.0 %	13.6 %	4.0 %	15.0 %
Acute nephritic syndrome	6.0 %	19.0 %	ND	ND	15.0 %	ND	12.0 %	ND
Acute kidney injury (AKI)	6.0 %	ND	25.4 %	28.9 %	ND	31.8 %	41.0 %	31.0 %
Chronic nephritic syndrome	ND	ND	4.6 %	4.7 %	ND	ND	ND	ND
CRF (or CKD)[a]	12.0 %	ND	18.9 %	16.2 %	23.0 %	11.5 %	9.0 %	19.0 %
Asymptomatic urinary abnormality	5.1 %	2.0 %	12.1 %	10.9 %	7.0 %	7.6 %	1.0 %	5.0 %
Macroscopic hematuria	ND	ND	0.7 %	0.7 %	ND	ND	ND	ND
Hypertensive nephrosclerosis	ND	ND	1.8 %	2.3 %	ND	2.1 %	ND	ND
Others	ND	ND	ND	ND	ND	3.4 %	ND	ND
Unknown	ND	ND	ND	ND	ND	4.2 %	ND	ND

ND not determined

[a] Chronic renal failure or chronic kidney disease

cholesterol acyltransferase (LCAT) deficiency in 1 case, cholesterol embolism in 5 cases, and IgG4-related renal injury in 12 cases.

Of the primary glomerular disease including IgA nephropathy suffered by the elderly patients, membranous nephropathy ($n = 485$, 38.5 %) was the most frequent

Table 12 Nephrotic syndrome in the elderly Japanese

Authors	Present study		Uezono		Komatsuda		Ozono		Sato	
Reported year	2012		2006		1993		1993		1987	
Study period	2007–2011		2000–2004		1979–1990		1971–1989		1958–1985	
Type of registry	Nation wide		Single center (Miyazaki)		Single center (Akita)		Single center (Nagasaki)		Single center (Tohoku)	
Total cases	10218		406		2088		ND		ND	
Elderly cases[a]	2802		61		247		ND		ND	
	≥65 years old		≥65 years old		≥65 years old		≥60 years old		≥60 years old	
Nephrotic cases (%):	1160	41.4	27	44.3	88	35.6	90	ND	87	ND
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
Primary disease										
IgA nephropathy (IgAN)	48	4.1	2	7.4	6	6.8				
Membranous nephropathy	365	31.5	4	14.8	35	39.8	26	28.9	30	34.5
Minimal change nephrotic syndrome	146	12.6	5	18.5	9	10.2	6	6.7	7	8.0
Focal segmental glomerulosclerosis	68	5.9	6	22.2	5	5.7	1	1.1		
Membranoproliferative glomerulonephritis type (I/III)	51	4.4			3	3.4	8	8.9	7	8.0
Crescentic glomerulonephritis	10	0.9			3	3.4	1	1.1	1	1.1
Mesangial proliferative glomerulonephritis except for IgA nephropathy	17	1.5			4	4.5	12	13.3	12	13.8
Other/unclassifiable	13	1.1			3	3.4	1	1.1		
Subtotal cases	718	61.9	17	63.0	68	77.3	55	61.1	57	65.5
Secondary disease	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Diabetic nephropathy	115	9.9	3	11.1	7	8.0	8	8.9	12	13.8
Amyloidosis	88	7.6	2	7.4	9	10.2	14	15.6	9	10.3
Lupus nephritis	18	1.6								
Infection-related nephropathy	17	1.5								
Nephrosclerosis	17	1.5	3	11.1						
Purpura nephritis	16	1.4			1	1.1				
MPO-ANCA-positive nephritis	19	1.6								
PR3-ANCA-positive nephritis	1	0.1			1	1.1				
Alport syndrome	1	0.1								
Thrombotic microangiopathy	1	0.1								
No conclusive diagnoses									4	4.6
Others	149	12.8	2	7.4	2	2.3	13	14.4	5	5.7
Subtotal cases	442	38.1	10	37.0	20	22.7	35	38.9	26	29.9

histological type, followed by IgA nephropathy ($n = 293$, 23.3 %), minor glomerular abnormalities ($n = 156$, 12.4 %), FSGS ($n = 99$, 7.9 %), and MPGN types I and III ($n = 75$, 6.0 %). A comparison with the control group showed that membranous nephropathy, MPGN type I and III, MPO-ANCA-positive nephritis, diabetic nephropathy, nephrosclerosis, and amyloid nephropathy were more frequent in the elderly ($P < 0.001$), and IgA nephropathy, minor glomerular abnormalities, lupus nephritis, and thin basement membrane disease were less frequent ($P < 0.001$) (Table 3).

Classification of the pathogenesis and histopathology of the elderly population

The pathological diagnoses of the elderly patients are shown in Table 4. More than half of the patients (59.5 %) presenting with nephrotic syndrome were found to have primary glomerular disease. Diabetic nephropathy was the second most common finding within the nephrotic group, but it displayed a much lower incidence (9.7 %). On the other hand, more than half of the elderly Japanese patients

(54.7 %) with RPGN were diagnosed with MPO-ANCA-positive nephritis. In contrast, approximately one-third of the patients who underwent renal biopsy because of a slowly progressive decline in their renal function exhibited findings of primary glomerular disease, IgA nephropathy, or hypertensive nephropathy (Table 4).

As for the histopathologic diagnoses shown in Table 5, the initial pathological findings were membranous nephropathy in nephrotic syndrome, mesangial proliferative glomerulonephritis in chronic nephritic syndrome, crescentic glomerulonephritis in rapidly progressive glomerulonephritis, and endocapillary proliferative glomerulonephritis in acute nephritic syndrome and mesangial proliferative glomerulonephritis in recurrent or persistent hematuria (Table 5).

Nephrotic syndrome in the elderly and very elderly Japanese patients

As for nephrotic syndrome, the elderly accounted for 1160 patients of the 2753 nephrotic syndrome patients (42.4 %) registered in Japan. In addition, nephrotic syndrome was the most frequent indication for biopsy in both the elderly (36.3 %) and very elderly (50.7 %) (Table 1). Membranous nephropathy ($n = 365$, 31.5 %; $n = 45$, 28.1 %) was the most frequent histopathological type in the elderly and very elderly, followed by minimal change nephrotic syndrome ($n = 146$, 12.6 %; $n = 19$, 11.9 %), diabetic nephropathy ($n = 115$, 9.9 %; $n = 10$, 6.3 %), amyloid nephropathy ($n = 88$, 7.6 %; $n = 19$, 11.9 %), and focal segmental glomerulosclerosis ($n = 68$, 5.9 %; $n = 12$, 7.5 %) (Tables 6, 7). A comparison with the control group found that membranous nephropathy, MPGN types I and III, and amyloid nephropathy were more frequent in the elderly ($P < 0.001$), whereas minimal change nephrotic syndrome, lupus nephritis ($P < 0.001$), and IgA nephropathy ($P = 0.006$) were less common (Table 6).

Rapidly progressive nephritis in elderly patients

In RPGN, elderly patients accounted for 432 of the 732 RPGN patients (59.0 %) registered in Japan. In addition, RPGN was the third and second most common indication for renal biopsy in the elderly and very elderly patients, respectively. ANCA-positive nephritis, especially MPO-ANCA-positive nephritis ($n = 245$, 56.7 %), was the most frequent histopathological type in the elderly, followed by systemic vasculitis ($n = 75$, 17.4 %). A comparison with the control group showed that the pauci-immune type (RPGN type III) was more frequent in the elderly ($P = 0.0235$), and type II ($P = 0.0026$) was less common (Table 8).

IgA nephropathy in the elderly patients

In contrast to nephrotic syndrome and RPGN, only 293 out of 3109 (9.4 %) IgA nephropathy patients were elderly (Table 9). In the elderly patients with IgA nephropathy, being male (64.5 %), advanced stage CKD (3b or worse) (44.7 %), nephrotic syndrome (9.2 %), and RPGN (6.8 %) were more common ($P < 0.001$). In addition, the proteinuria (daily proteinuria or the urinary protein/creatinine ratio), serum creatinine, and systolic blood pressure values of the patients were much higher than those of the controls (Table 10).

Proteinuria in the elderly: the discrepancy between daily proteinuria values and the urinary protein/creatinine ratio

There was a strong positive correlation between the urinary protein/creatinine ratio (g/gCr) and daily proteinuria (g/day) ($n = 4791$, $r = 0.796$, $P < 0.0001$); however, as shown in Fig. 1, there was a significant discrepancy between the urinary protein/creatinine ratio and daily proteinuria after the 7th decade of life. The mean urinary protein/creatinine ratio to daily proteinuria ratio was around 1.26–1.29 from the 3rd to 6th decade; however, it increased significantly to 1.46 in the 7th decade, 1.61 in the 8th decade, and 1.90 in the 9th decade and beyond (Kruskal–Wallis test, $P < 0.001$, Fig. 1).

Discussion and comments

To the best of our knowledge, this study constitutes the largest renal biopsy series of elderly (aged over 65 years) or very elderly (over 80 years old) in the world. We cannot exclude the possibility that the J-RBR is subject to sampling bias; however, an investigation of a larger cohort or a population-based analysis of the frequency of each renal disease utilizing our web-based system might reveal the actual frequencies of these diseases and their distributions throughout the age range. In addition, it is worth noting that a Web-based prospective registry system like the J-RBR could easily increase the number of participating institutions and enlarge the number of patients enrolled. Investigators could then analyze the registered data in real time and thus ensure that the present sample of patients in the J-RBR is representative of the nationwide frequency of renal diseases in Japan.

The present report revealed that among elderly and very elderly Japanese, renal biopsy is most commonly performed for nephrotic syndrome or AKI including RPGN. Similarly, nephrotic syndrome was the most common indication (37–64 %) for renal biopsy in elderly patients of

over 60 years old in South Korea, India, Italy, and Spain [12–15]. On the other hand, AKI including RPGN was the most common indication for renal biopsy (accounting for 31–41 % of cases) in elderly patients (over 65 years old) in the USA, west France, and Ireland [6, 7, 16, 17]. These findings reveal that renal biopsy is performed in the elderly all over the world to obtain significant diagnostic and prognostic information (Table 11). In agreement with this notion, renal biopsy was also performed for elderly patients with more advanced clinical abnormalities such as increased proteinuria, decreased GFR, and higher blood pressure, even in IgAN.

As for the pathogenesis and pathohistology of nephrotic syndrome, one-third of the elderly patients with nephrotic syndrome displayed primary membranous nephropathy, whereas minimal change nephrotic syndrome displayed a much lower frequency of about 12–13 % in the elderly and the very elderly. However, diagnosing minimal change nephrotic syndrome is useful as it allows the patient to be switched to steroid treatment. In addition, the frequency of amyloid nephropathy increased according to age from 2.3 % in the controls to 7.6 and 11.9 % in the elderly and very elderly, respectively. These findings support the previous results obtained in small studies from single centers in Japan [2–5] (Table 12) and other countries [12, 14, 15, 18–20].

As for RPGN, the results of this report are quite similar to those of a previous large retrospective cohort study from the Progressive Renal Diseases Research-RPGN study group [21] and other registry data regarding acute renal injury in older adults [6, 22, 23]. MPO-ANCA-positive nephritis with or without systemic vasculitis was the initial pathogenesis of RPGN in the elderly and the very elderly in this study. New guidelines for the treatment of RPGN targeting MPO-ANCA-positive nephritis have been proposed by the RPGN study group [24]. A prospective Web registry-based study examining the treatment and outcomes of RPGN and vasculitis has started, which might resolve the issues regarding the treatment of MPO-ANCA-positive nephritis with or without systemic vasculitis in the elderly [25]. Based on these findings, optimal therapeutic guidelines for RPGN in the elderly may be reported in future.

The present report revealed that IgA nephropathy in the elderly had the different gender background (the male-to-female ratio was 1.82:1) and more advanced clinical stage. On the other hand, there were no significant differences between the sexes in the controls. This finding was quite similar to the previous nationwide reports on IgA nephropathy in adult Japanese describing a male-to-female ratio from 1:1 in 660 cases to 1:0.9 in 502 cases [9, 26]. Concerning the gender background in this Japanese registry, renal biopsies were performed more frequently in males (the male-to-female ratio was 1.32:1), similar to other nationwide registry studies in adults (the male-to-female ratio has been described as 1.39:1 to 1.56:1), however [14, 15]. There were also differences in the male-to-female ratio of the elderly in the past reports (from 1.08:1 to 6.22:1) [12, 13, 15–17]. We could not exclude physician biases influencing the indications for renal biopsies in the elderly because there was a male predominance of the main clinical syndromes even in the elderly with IgA nephropathy [15]. In this regard, ongoing prospective cohort study of IgA nephropathy (J-IGACS study on J-RBR) may resolve the issues of gender and aging for the clinical progression of Japanese IgA nephropathy in the future.

In this study, we detected a discrepancy between daily proteinuria values and the urinary proteinuria/creatinine ratio in the elderly. This finding has important implications for the assessment of glomerular injuries in the elderly. Proteinuria is overestimated by the urinary proteinuria/creatinine ratio in the elderly because of the decreased excretion of urinary creatinine brought about by the reduction of muscle mass that occurs during aging [27, 28]. Thus, the degree of the overestimation of proteinuria by the proteinuria/creatinine ratio might increase with age. In addition, the progressive decline in GFR that occurs during aging, i.e., the decrease in the number of nephrons with age, should be considered when assessing the amount of protein lost from a single nephron in elderly patients. Daily proteinuria might underestimate the protein lost by a single nephron in the elderly. In the future, studies assessing proteinuria should resolve this issue regarding the early diagnosis and treatment of intractable glomerular diseases in the elderly.

In conclusion, renal biopsy is a valuable diagnostic tool, even in elderly and very elderly Japanese patients. In the future, modified clinical guidelines for elderly patients with renal disease should be developed.

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Conflicts of interest None of the authors has any conflicts of interest to disclose for this article.

Appendix 1

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