In Reply to "The Spectrum of Renal Abnormalities in Mitochondrial Disorders Is Broad"

The Authors Reply: We sincerely thank Dr. Finsterer for his interest in our study of mitochondrial nephropathy and would like to comment on the points raised as follows. At first, as pointed out by him,[1] heteroplasmy rates in mitochondrial DNA (mtDNA) is a key factor that affects clinicopathologic features and prognosis of mitochondrial diseases caused by mtDNA mutation. Therefore, we recognize that the lack of data on heteroplasmy rates is a limitation of our study and has already been mentioned likewise in the Discussion section of the original paper.[2] Here, we take this opportunity to further express our thoughts on the same. In the case of mitochondrial nephropathy caused by mtDNA mutation, it is highly expected that heteroplasmy rates of the kidney tissue should be associated with its clinicopathologic features and prognosis. However, in general routine practice, genetic tests are rarely performed using kidney tissue. We have started investigating heteroplasmy rates using not only blood but also cells from urine droplets or kidney tissue obtained by kidney biopsy for genetic diagnosis in cases of mitochondrial nephropathy caused by mtDNA mutation. Here, we presented data of 3 such cases with m.3243 A>G mutation (Table 1). The table suggests that the heteroplasmy rate of the blood cells is not equivalent to that of the kidney tissue. It also suggests that the heteroplasmy rate of the cells in the urine droplet does not match that of the kidney tissue. In addition, we hypothesize that the heteroplasmy rate is likely to be different in each cell type making up the kidney, which may determine the characteristics of each case of mitochondrial nephropathy. For example, when the rate of heteroplasmy is high in the cells of the proximal tubules, it is expected that the case presents with Fanconi syndrome. Similarly, when this rate is high in the glomerular epithelial cells or renal arteries/arterioles, it may be a case of focal segmental glomerulosclerosis or nephrosclerosis, respectively. These are the hypotheses that should be considered in the future.

Furthermore, the copy number of mtDNA is thought to play an important role in determining the phenotype of mitochondrial diseases,[3,4] as has been pointed out.[1] However, the data of the mtDNA copy number were not collected in this study. The presence of renal cysts, nephrolithiasis, or neoplasm was also not included in the yes/no questions in our study questionnaire. Nevertheless, the questionnaire had open-ended boxes to fill in other renal abnormalities or complications, and these were rechecked by us. One case of renal cyst and another of nephrolithiasis were observed, but because both cases did not have a genetic diagnosis, they had not been included in the analysis of mitochondrial nephropathy. In 1 case with multiple deletions in mtDNA, esophageal epidermoid carcinoma was noted. These counts were based on free descriptions, and it is quite possible that the analysis was inadequate. In response to his question, we attempted to do an additional analysis on the presence of hematuria in the urinary sediment tests; of the 81 cases of mitochondrial nephropathy, 26 (32.1%) had hematuria. Along with that, of the 63 patients with m.3243A>G mutation, 19 (30.2%) had hematuria. Because glomerular lesions caused by mitochondrial disease usually do not cause tissue changes resulting in hematuria, urolithiasis/nephrolithiasis may have been the cause of such high incidents of hematuria. Although there is a possibility that drugs used in the cases analyzed in our study induced their renal damage (especially tubulointerstitial nephropathy), the data necessary to answer this issue accurately have not been collected.

Finally, it is difficult to differentiate between secondary mitochondrial nephropathy (which is caused by mitochondrial cardiomyopathy or mitochondrial diabetes) and primary mitochondrial nephropathy (which is caused by genetically defective oxidative phosphorylation function in the cells of the nephron). This was discussed in the Discussion section of the original paper,[2] but here we discuss the same in detail. As mentioned in the paper, it is technically possible to measure mitochondrial respiratory chain enzyme activity in the renal tissue; however, collecting renal tissue samples for enzyme activity measurement is not possible in actual clinical

Table 1. Differences in heteroplasmy rates of mtDNA in the blood cell, kidney tissue, and cells in urine droplet

Case no.	Mutation	Renal pathology	Heteroplasmy rates (%)		
			Blood cell	Kidney	Urine droplet
Case 1	m.3243 A>G	Nephrosclerosis	19.7	74.6	53.4
Case 2	m.3243 A>G	Nephrosclerosis	29.2	74.5	65.3
Case 3	m.3243 A>G	Diabetic nephropathy	0	0	29.0

mtDNA, mitochondria DNA.
Cases 1 and 2 were included in the analysis of the original paper,[2] whereas, case 3 has been newly added. This study was approved by the independent ethics committee and institutional review board of Chiba Children's Hospital (number 2020-015). The study was conducted in accordance with the principles of the Declaration of Helsinki.

practice and is generally not recommended to all the patients. One possible method could be to examine expression of mitochondrial respiratory chain enzyme proteins in renal biopsy tissue, as it has been reported.[5] In addition, the analysis of heteroplasmy rates of mtDNA in the renal tissue may give an answer in a few of these cases. For example, in case 3 illustrated in Table 1, renal abnormality should not be caused by mitochondrial respiratory chain dysfunction of the renal tissues directly because of the gene mutation. Because a much smaller amount of the renal tissue sample is sufficient to perform this analysis than that needed for the measurement of mitochondrial respiratory chain activity, it could be one way to distinguish between the 2 pathologies. This problem could be solved by examining renal oxygen consumption by functional MRI or biomarkers in the urine, in the future.

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Toshiyuki Imasawa[1] and Kei Murayama[2]

[1]Department of Nephrology, National Hospital Organization Chiba-Higashi National Hospital, Chiba-city, Chiba, Japan and [2]Center for Medical Genetics, Department of Metabolism, Chiba Children's Hospital, Midori-ku, Chiba, Japan

Correspondence: Toshiyuki Imasawa, Department of Nephrology, National Hospital Organization Chiba-Higashi National Hospital, 673 Nitona-cho, Chuoh-ku, Chiba-city, Chiba 206-8712, Japan. E-mail: imasawa@nifty.com

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