Synergistic effect of proteinuria on dipstick hematuria-related decline in kidney function: The Japan Specific Health Checkups (J-SHC) Study.

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Abstract

Background: The effect of isolated hematuria without proteinuria on kidney function decline, and the modification by the severity of proteinuria in general population are not fully elucidated.

Methods: Participants were included in the Japan Specific Health Checkups Study between 2008 and 2014. The exposure of interest was the frequency of dipstick hematuria during the observation. In each proteinuria frequency category (non-, occasional, persistent), hematuria-related decline in the eGFR rate was examined by analysis of covariance (ANCOVA). eGFR decline trajectories were also assessed using mixed-effects models.

Results: Among the 552,951 participants, 146,753 (26.5%) had hematuria, and 56,021 (10.1%) and 8,061 (1.5%) had occasional and persistent proteinuria, respectively. During the median follow-up of 3.0 years, annual change in eGFR decline in participants with hematuria was significantly faster than in those without hematuria (mean [95% confidence interval]: −0.95 [−0.98 to −0.92] vs −0.86 [−0.87 to −0.84] mL/min/1.73 m2/year; P <0.001). In ANCOVA, the hematuria-related annual eGFR decline rate increased as proteinuria frequency categories increased (differences in annual eGFR decline rate between participants with and without hematuria: 0.08 [0.06 to 0.09] in participants with non-proteinuria category, 0.17 [0.15 to 0.18] in occasional proteinuria category, and 0.68 [0.65 to 0.71] mL/min/1.73 m^2/year in persistent proteinuria category; P for interaction <0.001). Similar results were obtained by the linear mixed-effect model.

Conclusions: Proteinuria has a synergistic effect on dipstick hematuria-related decline in kidney function. Among the general population without proteinuria throughout the observational period, the “isolated hematuria”-related eGFR decline was statistically significant but the difference was small.

Keywords: eGFR decline, hematuria, proteinuria, sex differences

Introduction

Urinalysis is a useful method for detecting kidney diseases at an early stage [[1, 2]]. Previous observational studies have shown that abnormalities in urinalysis in the general population are associated with poor kidney outcomes [[3-6]]. Among these abnormalities in urinalysis, including hematuria and proteinuria, asymptomatic hematuria has generally been regarded as having little effect on kidney prognosis. In particular, patients with hematuria but not proteinuria rarely show progression of chronic kidney disease (CKD) [[4, 5, 7]]. In contrast, it is widely accepted that proteinuria is an independent risk factor for deterioration of kidney function and is used as a marker of kidney prognosis in clinical settings [[3]].

Current reports focus on the fact that hematuria has been indicated as a prognostic marker of deteriorating kidney function [[5, 8-10]]. Especially in patients with immunoglobulin A (IgA) nephropathy, microscopic hematuria was shown to be a significant risk factor for the progression of CKD [[9, 10]], and remission of hematuria as well as proteinuria was associated with favorable kidney outcomes [[11]]. However, some reports describe hematuria as associated with kidney disease progression only in patients with persistent proteinuria but not in non-proteinuric patients [[8, 12]].

Therefore, the clinical implications of hematuria remain controversial, and the actual impact of "isolated hematuria without proteinuria" on decline in kidney function is unknown. We speculate that hematuria itself has minimal impact on decline in kidney function and that proteinuria is a potential

modifier of hematuria-related decline in kidney function.

The aim of this study was to elucidate the effect of proteinuria modification on hematuria-related decline in eGFR and estimate the actual impact of isolated hematuria without proteinuria on kidney function among large, general, population-based databases.

Materials and methods

Data sources and study participants

This longitudinal study was based on databases created for the Japan Specific Health Check and Guidance System (J-SHC Study). The age range of all participants was 40 to 74 years. Participants underwent an annual health checkup between 2008 and 2014. The details of the health checkup system have been previously described[13]. The exclusion criteria included the following: 1) less than two measurements of eGFR and/or urinalysis; 2) higher than 150 mL/min/1.73 m^2 of eGFR (overestimated probably due to severe muscle mass loss); and 3) less than 9 months in observational periods.

Measurements and exposures

During the follow-up period (2008–2014), visits to the annual medical check-up program were voluntary. Each participant had a different visit frequency and interval. All participants completed a self-administered questionnaire to document their medical history, current medications, smoking habits, and regular exercise

habits (more than 30 min at least 2 days a week or not). Blood pressure measurements and blood and urine samples were collected at each participant's local medical institute after overnight fasting for more than 10 h according to the health check program.

Dipstick hematuria and proteinuria were evaluated using the following five scales: (−), (±), (1+), (2+), and (3+). The presence of hematuria was defined as (1+) or more than once during the observational period. Proteinuria categories was divided into three groups according to the following frequencies: "Persistent proteinuria", (1+) or more was present persistently during the observational periods; "Occasional proteinuria", (1+) or more was present occasionally during the observational period; and "Non-proteinuria".

Outcomes

The outcome of this study was the annual eGFR decline rate that was determined using longitudinal eGFR data over the observational period. The eGFR was calculated using the following equation: eGFR (mL/min/1.73 m^2) =194 ×serum creatinine (mg/dL)$^{-1.094}$ ×age (years)$^{-0.287}$ ×0.739 (for females), as previously described[14].

Statistical analysis

Baseline data are reported as medians with interquartile ranges for continuous variables and numbers with percentages for categorical variables. Because of the large number of participants, standardized

mean difference (SMD) >0.2 was considered as a clinically relevant difference instead of a *P* value <0.05. The annual change in eGFR decline estimated by the ordinary least squares method was used for analysis of covariance (ANCOVA). Baseline data including age, sex, body mass index (BMI), current smoking status, systolic blood pressure, eGFR, hemoglobin A1c (HbA1c), uric acid, antihypertensive drugs, antidiabetic drugs, heart diseases, stroke, and kidney diseases were used as potential confounders to adjust for the covariates in the ANCOVA. To examine the trend of the prevalence of hematuria among proteinuria categories, we used the Cochran-Armitage test.

We also used an unadjusted restricted cubic spline curve (considering repeated measure data) and a multivariate linear mixed effect model with a random intercept and random slope to estimate the eGFR decline trajectories with age. As there was a large difference in the prevalence of hematuria by sex, we performed stratified analyses by sex for the above-mentioned statistics.

All statistical analyses were performed using IBM SPSS version 25.0 (SPSS Institute, Tokyo, Japan) and R software version 3.6.1 (R Foundation, Vienna, Austria).

Results

The baseline characteristics of this study

Among 933,490 participants from 27 districts, participants without multiple measurements for eGFR and/or urinalysis, and those with eGFR >150 mL/min/1.73 m^2 and/or an observational period less than 0.75 years (9 months) were excluded, and the remaining 552,951 were included in this study (Figure 1). Because annual eGFR decline rates among participants with an observational period of less than 9 months were unreliably decreased compared with those among other participants, we excluded them from this study (Figure S1). The mean [interquartile range] observation period was 3.0 [1.9 to 4.1] years. The baseline characteristics stratified by hematuria and proteinuria categories are summarized in Tables 1 and 2, respectively. The mean [interquartile range] baseline eGFR was not different between participants with hematuria (74.7 [63.9 to 84.7] mL/min/1.73 m^2) and those without hematuria (74.7 [64.6 to 85.3] mL/min/1.73 m^2) (SMD = 0.035). Hematuria was associated with a higher proportion of female participants and a higher frequency of proteinuria (Table 1). The overall prevalence of hematuria was 26.5% and was approximately two-fold higher in females (34.2%) than in males (15.9%). Despite the large difference of prevalence of hematuria between male and female participants, an incremental trend in prevalence of hematuria with increased proteinuria frequency was about two-fold higher in participants with persistent proteinuria than in those without proteinuria; this result was similarly observed in both sexes (Figure S2, *P* for interaction =0.603). No other differences were observed between the participants with

and without hematuria in either sex (Table S1). As shown in Table 2, higher proteinuria frequency was associated with a higher proportion of males, higher BMI and blood pressure, more dyslipidemia, higher HbA1c, lower eGFR, higher serum creatinine, higher uric acid, and more history of kidney diseases (SMD >0.2).

Hematuria-related annual eGFR decline rate estimated by ANCOVA

First, we examined the effect of hematuria on the annual eGFR decline rate in the entire cohort. As shown in Table 3, participants with hematuria showed a significantly faster decline in eGFR than those without hematuria did. Even after full adjustment for clinically relevant factors (Model 3), this association remained statistically significant. The mean (95% confidence interval: CI) annual eGFR decline rate in participants with hematuria was −0.99 (−1.02 to −0.97) mL/min/1.73 m^2/year and that in participants without hematuria was −0.84 (−0.86 to −0.82) mL/min/1.73 m^2/year; difference: 0.16 (0.12 to 0.19) mL/min/1.73 m^2/year.

In ANCOVA model, the annual eGFR decline rate was greater in the hematuria group than in the non-hematuria group for all proteinuria categories (Figure 2A). The mean (95% CI) annual eGFR decline rates in participants with and without hematuria were −0.88 (−0.91 to −0.84) vs −0.80 (−0.82 to −0.78) in participants with non-proteinuria category, −1.23 (−1.31 to −1.15) vs −1.06 (−1.12 to −1.00) in occasional proteinuria category, and −3.29 (−3.49 to −3.10) vs −2.64 (−2.80 to −2.41) mL/min/1.73

m^2/year in persistent proteinuria category. With more severe proteinuria categories, the difference in the annual eGFR decline rates between the hematuria and non-hematuria groups gradually increased (Figure 2A). The differences in annual eGFR decline rate between participants with and without hematuria were 0.08 (0.06 to 0.09) in participants with non-proteinuria category, 0.17 (0.15 to 0.18) in occasional proteinuria category, and 0.68 (0.65 to 0.71) mL/min/1.73 m^2/year in persistent proteinuria category (*P* for interaction <0.001). That means the participants with hematuria had 8.8 (7.6 to 10.0)%, 13.6 (13.1 to 13.9)% and, 20.2 (19.9 to 20.6)% faster annual eGFR decline rates as compared to those without hematuria in non-proteinuria, occasional proteinuria, and persistent proteinuria category, respectively. To examine sex difference in this effect, these associations were also examined in male and female (Figure 2, B and C). This effect modification of proteinuria on the hematuria-related annual eGFR decline rate was significant in male participants (*P* for interaction <0.001) but not in female participants (*P* for interaction =0.43). Furthermore, to examine the effect of age, we performed a stratified analysis by age (Figure 2D: younger age: <60 years and Figure 2E: older age: 60 years or older). The effect of proteinuria on the hematuria-related annual eGFR decline rate in older participants (≥60 years) was significantly greater than those in younger participants (<60 years) (*P* for interaction =0.085).

eGFR decline trajectories with and without hematuria estimated by the mixed effect model

Considering repeat measure eGFR data, restricted cubic spline curve was used to estimate the

unadjusted eGFR decline trajectories with aging between hematuria and non-hematuria groups (Figure 3). The trajectory of eGFR decline in the hematuria group was steeper than that in the non-hematuria group. This difference was more obvious in males than in females (Figure 3, B and C, interaction *P* for sex <0.001). As the proteinuria category became more severe, the eGFR decline trajectory became steeper and the difference in the eGFR decline slope between the hematuria and non-hematuria groups was incrementally increased (Figure 3, D–F, interaction *P* for proteinuria <0.001).

In the multivariate linear mixed effect model (random intercept and slope), the annual eGFR decline rate in the hematuria group (mean ± standard error [SE], −0.62 ± 0.004 mL/min/1.73 m^2/year) was significantly greater than that in the non-hematuria group (−0.57 ± 0.002 mL/min/1.73 m^2/year) (Figure 4A). The difference in annual eGFR decline rate between participants in the hematuria and non-hematuria groups was 0.062 ± 0.009 mL/min/1.73 m^2/year (*P* <0.001). In the stratified analyses by sex, male sex revealed that the annual eGFR decline rate in the hematuria group (mean ± SE −0.75 ± 0.008 mL/min/1.73 m^2/year) was significantly greater than that in the non-hematuria group (−0.61 ± 0.003 mL/min/1.73 m^2/year) (Figure 4B, *P* <0.001). Similarly, female sex revealed that the annual eGFR decline rate in the hematuria group (−0.57 ± 0.004 mL/min/1.73 m^2/year) was significantly greater than that in the non-hematuria group (−0.53 ± 0.003 mL/min/1.73 m^2/year) (Figure 4C, *P* <0.001). However, the difference in annual eGFR decline rate with and without hematuria in males (0.14 ± 0.015 mL/min/1.73 m^2/year) was significantly greater than that in females (0.05 ± 0.01 mL/min/1.73 m^2/year)

(interaction *P* for sex =0.09). In terms of the annual eGFR decline rate in each proteinuria category, the non-proteinuria category revealed that the annual eGFR decline rate in the hematuria group (−0.59 ± 0.004 mL/min/1.73 m^2/year) was significantly greater than that in the non-hematuria group (−0.55 ± 0.002 mL/min/1.73 m^2/year) (Figure 4D, *P* <0.001). In the occasional proteinuria category, the annual eGFR decline rate in the hematuria group (−0.71 ± 0.01 mL/min/1.73 m^2/year) was significantly greater than that in the non-hematuria group (−0.68 ± 0.008 mL/min/1.73 m^2/year) (Figure 4E, *P* =0.03). In the persistent proteinuria category, the annual eGFR decline rate in the hematuria group (−1.12 ± 0.04 mL/min/1.73 m^2/year) was significantly greater than that in the non-hematuria group (−0.99 ± 0.03 mL/min/1.73 m^2/year) (Figure 4F, *P* =0.01). Notably, the difference in annual eGFR decline rate with and without hematuria increased as the proteinuria category became more severe (non-proteinuria; 0.04 ± 0.009, occasional proteinuria; 0.03 ± 0.02, and persistent proteinuria; 0.12 ± 0.09 mL/min/1.73 m^2/year, interaction *P* for proteinuria category <0.001). Figure 5 shows the multivariate linear mixed effect model (random intercept and slope) in male and female. In male gender, non-proteinuria category revealed that the annual eGFR decline rate in hematuria group (−0.68 ± 0.008 mL/min/1.73 m^2/year) was significantly greater than that in non-hematuria group (−0.59 ± 0.003 mL/min/1.73 m^2/year) (Figure 5A, *P* <0.001). In occasional proteinuria category, the annual eGFR decline rate in hematuria group (−0.85 ± 0.02 mL/min/1.73 m^2/year) was significantly greater than that in non-hematuria group (−0.74 ± 0.011 mL/min/1.73 m^2/year) (Figure 5B, *P* <0.001). In persistent proteinuria category, the annual eGFR decline

rate in hematuria group (−1.18 ± 0.05 mL/min/1.73 m^2/year) was significantly greater than that in non-hematuria group (−1.01 ± 0.04 mL/min/1.73 m^2/year) (Figure 5C, *P* =0.02). The difference in annual eGFR decline rate with and without hematuria increased as the proteinuria category became more severe (non-proteinuria; 0.09 ± 0.02, occasional proteinuria; 0.11 ± 0.04, and persistent proteinuria; 0.13 ± 0.11 mL/min/1.73 m^2/year, interaction *P* for proteinuria category <0.001). On the other hand, in female gender, non-proteinuria category revealed that the annual eGFR decline rate in hematuria group (−0.56 ± 0.005 mL/min/1.73 m^2/year) was significantly greater than that in non-hematuria group (−0.52 ± 0.004 mL/min/1.73 m^2/year) (Figure 5D, *P* <0.001). In occasional proteinuria category, the difference in the annual eGFR decline rate between hematuria group (−0.64 ± 0.01 mL/min/1.73 m^2/year) and non-hematuria group (−0.63 ± 0.01 mL/min/1.73 m^2/year) were not statistically significant (Figure 5E, *P* =0.81). In persistent proteinuria category, the annual eGFR decline rate in hematuria group (−0.99 ± 0.06 mL/min/1.73 m^2/year) was greater than that in non-hematuria group (−0.87 ± 0.06 mL/min/1.73 m^2/year) but the difference was not statistically significant (Figure 5F, *P* =0.29). The difference in annual eGFR decline rate with and without hematuria in non-proteinuria, occasional proteinuria, and persistent proteinuria group were 0.04 ± 0.011, 0.005 ± 0.038, and 0.09 ± 0.16 mL/min/1.73 m^2/year, respectively (interaction *P* for proteinuria category =0.31). This synergistic effect of proteinuria on hematuria-related annual eGFR decline in male was greater than that in females (interaction *P* for gender =0.087).

Discussion

The present study aimed to determine the actual impact of "isolated hematuria itself" on kidney function. To the best of our knowledge, this is the first report describing the synergistic effect of proteinuria on hematuria-related eGFR decline. Among participants without proteinuria, the effect of hematuria on kidney dysfunction was statistically significant (the difference in eGFR decline slope estimated by ANCOVA: mean 0.077, 95% CI [0.025–0.118] mL/min/1.73 m^2/year, and that by a linear mixed-effect model: mean ± standard error, 0.062 ± 0.004 mL/min/1.73 m^2/year, P <0.001) but the difference was small. In this study, the cause of hematuria and proteinuria includes several diseases such as primary glomerulonephritis, thin basement membrane disease, ADPKD, and non-glomerular diseases (kidney stone, cystitis). Therefor the impact of hematuria on kidney function would be different among the cause of primary diseases. Although the mechanism of synergistic effect of proteinuria and hematuria on kidney function is not uncertain, the presence of both proteinuria and hematuria concurrently suggests a more significant kidney pathology and may indicate more severe kidney damage. The combination of these two findings can be seen in conditions such as glomerulonephritis, vasculitis, and lupus nephritis. These conditions typically involve inflammation and damage to the glomeruli. Notably, there was a large difference in the prevalence of hematuria between males (15.9%) and females (34.2%), implying that the positivity of dipstick hematuria in this study included hematuria of non-glomerular origin, such as hematuria of gynecological origin. Previous reports described that the prevalence of hematuria in females

was about two- to three-fold higher than that in males [[3, 5, 6]]. Iseki et al. reported that the prevalence of hematuria was 3.48% in males and 12.3% in females. Similarly, Kim et al. and Chadban et al. reported rates of 3.9% and 2.0% in males and 9.7% and 7.2% in females, respectively. These results are consistent with our results (Figure S2). The potential causes of hematuria vary by sex: prostatic disorders in males [[15, 16]] and menstruation, ovarian, and uterine disorders in females [[17-20]]. Indeed, we examined the prevalence of hematuria by age group in males and females (Figure S3). Interestingly, the prevalence of hematuria gradually increased with increasing age in males but remained unchanged across age in females. These results suggest that the increased prevalence of hematuria in elderly male participants is associated with prostatic disorders and that menstruation has little effect on hematuria prevalence in younger female participants. Because of the large difference in the prevalence of hematuria by sex, we also examined the effect of hematuria on kidney dysfunction (Figure 5). As expected, the association between hematuria and kidney dysfunction was stronger in males than in females, suggesting that a higher prevalence of hematuria in females would include non-glomerular origin as compared with males. However, the impact of isolated hematuria without proteinuria on kidney dysfunction was still very small even in male participants (difference in annual eGFR decline rate between hematuria and non-hematuria among participants without proteinuria: mean ± SE 0.093 ± 0.009 mL/min/1.73 m^2/year).

Previous studies have shown that isolated microscopic hematuria at baseline (at the start of observation) was associated with poor kidney outcomes [[4, 21, 3, 5, 7, 22]]. Some of these studies

reported that proteinuria accelerated hematuria-related kidney dysfunction [[4, 3, 5]]. Notably, proteinuria and hematuria also reported to have a synergistic effect on mortality [[23, 24]]. These results are consistent with our results. However, these studies did not account for proteinuria during follow-up periods, because some patients with isolated microscopic hematuria eventually become proteinuric during follow-up. Therefore, these studies did not accurately assess the impact of "isolated hematuria without proteinuria" on kidney dysfunction. Using time-varying exposure to urinalysis abnormalities during the follow-up, this study revealed the actual impact of isolated hematuria without proteinuria and the synergistic effect of proteinuria on hematuria-related progression of CKD.

This study had several limitations. First, the positivity of dipstick hematuria is not completely consistent with microscopic hematuria, in which red blood cells (RBC) in urine are confirmed by light microscopy (generally urinary RBC >5/high power field). Because the dipstick urine test detects the reaction between hemoglobin and peroxidase activity [[25]], various factors (antioxidants, bacteremia, pH, etc.) can result in a false-positive or negative in this test [[26-28]]. We also have no data on urine specific gravity in this cohort. Therefore, it was not possible to evaluate dipstick hematuria accurately taking urine specific gravity into consideration.. Second, participants with hematuria in this study included those with non-glomerular origin hematuria, especially in females. A two-fold higher prevalence of hematuria was observed in female participants as compared with male participants. This finding can be explained by the association between hematuria and kidney dysfunction in females that

was weaker than that in males. Third, different from other previous studies consisting of chronic glomerulonephritis (i.e. IgA nephropathy) [[8, 11]], this study is based on the general population including glomerular and non-glomerular disease other than chronic glomerulonephritis (i.e. hypertensive nephrosclerosis, diabetic kidney disease, and hematuria from non-glomerular diseases). Recently, the significance of remission of hematuria in the treatment of glomerulonephritis, especially IgA nephropathy, has been discussed, but the results of this study do not provide an answer to this question. This study focused on the significance of urinary occult blood on kidney function among the general population.

In conclusion, proteinuria has a synergistic effect on hematuria-related decline in kidney function. Among the general population, the effect of isolated hematuria without proteinuria on the decline in kidney function was statistically significant but the difference was small.

Compliance with Ethical Standards

Conflict of interest

The authors have declared that no conflict of interest exists.

Ethics Approval and Consent to Participate

All procedures involving human participants were carried out in accordance with the ethical standards of the institutional research committee at which the studies were conducted (Fukushima Medical University; IRB Approval Number #1485, #2771) and in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was conducted in accordance with the Ethical Guidelines for Medical and Health Research Involving Human Subjects that was enacted by the Ministry of Health, Labour and Welfare of Japan (http://www.mhlw.go.jp/file/06-Seisakujouhou-10600000-Daijinkanboukouseikagakuka/0000069410.pdf). In the context of these guidelines, investigators are not necessarily required to obtain informed consent. Instead, we provided public information concerning the study on our website (http://www.fmu.ac.jp/univ/sangaku/data/koukai_2/2771.pdf) and ensured that there were opportunities for the research participants to refuse the use of their personal information.

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Authors' Contributions

Research idea and study design: HT, ME, HY, TU, FF, MN, TK, MM, KS, KT; data acquisition: HY, KI, CI, KA, KY, TK, SF, IN, MKa, YS, TM, MKo, TW, KT; data analysis/interpretation: HT, ME, KT; statistical analysis: HT, ME, HY; supervision or mentorship: ME, KT. Each author contributed important intellectual content during manuscript drafting or revision and accepted accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work were appropriately investigated and resolved.

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References

1. Yamagata K, Iseki K, Nitta K, Imai H, Iino Y, Matsuo S et al. Chronic kidney disease perspectives in Japan and the importance of urinalysis screening. Clin Exp Nephrol. 2008;12(1):1-8. doi:10.1007/s10157-007-0010-9.
2. Imai E, Yamagata K, Iseki K, Iso H, Horio M, Mkino H et al. Kidney disease screening program in Japan: history, outcome, and perspectives. Clin J Am Soc Nephrol. 2007;2(6):1360-6. doi:10.2215/CJN.00980207.
3. Iseki K, Iseki C, Ikemiya Y, Fukiyama K. Risk of developing end-stage renal disease in a cohort of mass screening. Kidney Int. 1996;49(3):800-5. doi:10.1038/ki.1996.111.
4. Yamagata K, Takahashi H, Tomida C, Yamagata Y, Koyama A. Prognosis of Asymptomatic Hematuria and/or Proteinuria in Men. Nephron. 2002;91:34-42.
5. Kim H, Lee M, Cha MU, Nam KH, An SY, Park S et al. Microscopic hematuria is a risk factor of incident chronic kidney disease in the Korean general population: a community-based prospective cohort study. QJM. 2018;111(6):389-97. doi:10.1093/qjmed/hcy054.
6. Chadban SJ, Briganti EM, Kerr PG, Dunstan DW, Welborn TA, Zimmet PZ et al. Prevalence of kidney damage in Australian adults: The AusDiab kidney study. J Am Soc Nephrol. 2003;14(7 Suppl 2):S131-8. doi:10.1097/01.asn.0000070152.11927.4a.
7. Lee HM, Hyun JI, Min JW, Lee K, Kim YK, Choi EJ et al. The Natural Course of Biopsy-Proven Isolated Microscopic Hematuria: a Single Center Experience of 350 Patients. J Korean Med Sci. 2016;31(6):909-14. doi:10.3346/jkms.2016.31.6.909.
8. Yu GZ, Guo L, Dong JF, Shi SF, Liu LJ, Wang JW et al. Persistent Hematuria and Kidney Disease Progression in IgA Nephropathy: A Cohort Study. Am J Kidney Dis. 2020;76(1):90-9. doi:10.1053/j.ajkd.2019.11.008.
9. Goto M, Wakai K, Kawamura T, Ando M, Endoh M, Tomino Y. A scoring system to predict renal outcome in IgA nephropathy: a nationwide 10-year prospective cohort study. Nephrol Dial Transplant. 2009;24(10):3068-74. doi:10.1093/ndt/gfp273.
10. Le W, Liang S, Hu Y, Deng K, Bao H, Zeng C et al. Long-term renal survival and related risk factors in patients with IgA nephropathy: results from a cohort of 1155 cases in a Chinese adult population. Nephrol Dial Transplant. 2012;27(4):1479-85. doi:10.1093/ndt/gfr527.
11. Sevillano AM, Gutierrez E, Yuste C, Cavero T, Merida E, Rodriguez P et al. Remission of Hematuria Improves Renal Survival in IgA Nephropathy. J Am Soc Nephrol. 2017;28(10):3089-99. doi:10.1681/ASN.2017010108.
12. Gutierrez E, Zamora I, Ballarin JA, Arce Y, Jimenez S, Quereda C et al. Long-term outcomes of IgA nephropathy presenting with minimal or no proteinuria. J Am Soc Nephrol. 2012;23(10):1753-60. doi:10.1681/ASN.2012010063.

13. Tsuruya. K, Yoshida. H, Nagata. M, Kitazono. T, Hirakata. H, Iseki. K et al. Association of the triglycerides to high-density lipoprotein cholesterol ratio with the risk of chronic kidney disease: analysis in a large Japanese population. Atherosclerosis. 2014;233(1):260-7. doi:10.1016/j.atherosclerosis.2013.12.037.
14. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K et al. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis. 2009;53(6):982-92. doi:10.1053/j.ajkd.2008.12.034.
15. Gofrit ON, Katz R, Shapiro A, Yutkin V, Pizov G, Zorn KC et al. Gross hematuria in patients with prostate cancer: etiology and management. ISRN Surg. 2013;2013:685327. doi:10.1155/2013/685327.
16. Rastinehad AR, Ost MC, VanderBrink BA, Siegel DN, Kavoussi LR. Persistent prostatic hematuria. Nat Clin Pract Urol. 2008;5(3):159-65. doi:10.1038/ncpuro1044.
17. Chan. D, Ong. A, Schoenberg. M. Microscopic hematuria. N Engl J Med. 2003;349(13):1292-3. doi:10.1056/NEJM200309253491317.
18. Mohr. DN, Offord. KP, Owen. RA, 3rd. LJM. Asymptomatic microhematuria and urologic disease. A population-based study. JAMA. 1986;256(2):224-9.
19. Bradley MS, Willis-Gray MG, Amundsen CL, Siddiqui NY. Microhematuria in Postmenopausal Women: Adherence to Guidelines in a Tertiary Care Setting. J Urol. 2016;195(4 Pt 1):937-41. doi:10.1016/j.juro.2015.10.136.
20. Pillalamarri N, Shalom D, Sanidad S, Akerman M, Lind L, Winkler H. The prevalence of microscopic hematuria in a cohort of women with pelvic organ prolapse. Int Urogynecol J. 2015;26(1):85-90. doi:10.1007/s00192-014-2450-6.
21. Vivante. A, Afek. A, Frenkel-Nir. Y, Tzur. D, Farfel. A, Golan. E et al. Persistent Asymptomatic Isolated Microscopic Hematuria in Israeli Adolescents and Young Adults and Risk for End-Stage Renal Disease. JAMA. 2011;306(7):729-36. doi:10.1001/jama.2011.1141.
22. Iseki K. Evidence for asymptomatic microhematuria as a risk factor for the development of ESRD. Am J Kidney Dis. 2012;60(1):12-4. doi:10.1053/j.ajkd.2012.01.009.
23. Iseki K, Konta T, Asahi K, Yamagata K, Fujimoto S, Tsuruya K et al. Higher cardiovascular mortality in men with persistent dipstick hematuria. Clin Exp Nephrol. 2021;25(2):150-6. doi:10.1007/s10157-020-01971-z.
24. Iseki K, Konta T, Asahi K, Yamagata K, Fujimoto S, Tsuruya K et al. Association of dipstick hematuria with all-cause mortality in the general population: results from the specific health check and guidance program in Japan. Nephrol Dial Transplant. 2018;33(5):825-32. doi:10.1093/ndt/gfx213.
25. Pugia. MJ, Lott. JA, Wallace. JF, Cast. TK, Bierbaum. LD. Assay of creatinine using the peroxidase activity of copper-creatinine complexes. Clin Biochem. 2000;33(1):63-70.

doi:10.1016/s0009-9120(99)00086-7.
26. Ikeda K, Abe M, Masamoto I, Ishii C, Arimura E, Ushikai M et al. Comparison of dipstick and quantitative tests for proteinuria and hematuria in middle-aged, male Japanese employees: A single-center study. Health Sci Rep. 2021;4(2):e267. doi:10.1002/hsr2.267.
27. Pirkle JL, Palavecino EL, Freedman BI. Lactobacillus species can cause a false-positive test for hematuria on dipstick urinalysis. Am J Med. 2013;126(1):e3-4. doi:10.1016/j.amjmed.2012.07.017.
28. Levin. K, Engström. I. Inadequate hemolysis of erythrocytes on reagent strips at low pH causes false-negative readings. Clin Chem. 1984;30(11):1845-7.

Tables

Table 1. Baseline characteristic stratified by a hematuria category.

	Non-hematuria	Hematuria	SMD	Missing, n
Number of subjects	406,198	146,753		0
Male, n (%)	195,370 (48.1)	37,043 (25.2)	0.488	0
Age, years	64 (59–68)	65 (60–68)	0.091	0
BMI, kg/m^2	23.1 (21.1–25.2)	22.5 (20.6–24.7)	0.148	5,637
Observational periods, years	3.0 (1.9–4.1)	3.3 (2.0–4.3)	0.191	0
Systolic blood pressure, mmHg	128 (118–140)	128 (117–140)	0.033	2,450
Diastolic blood pressure, mmHg	77 (70–84)	76 (69–82)	0.099	2,527
Laboratory data				
Triglyceride, mg/dL	102 (74–147)	98 (71–138)	0.097	49
HDL cholesterol, mg/dL	60 (50–71)	62 (52–73)	0.119	19
LDL cholesterol, mg/dL	124 (105–145)	126 (106–147)	0.048	56
HbA1c, %	5.2 (5.0–5.5)	5.2 (5.0–5.5)	0.133	12,548
Serum creatinine, mg/dL	0.70 (0.60–0.80)	0.70 (0.60–0.80)	0.164	0
eGFR, mL/min/1.73m^2	74.7 (64.6–85.3)	74.7 (63.9–84.7)	0.035	0
Uric acid, mg/dL	5.2 (4.3–6.2)	4.8 (4.1–5.7)	0.217	14,588
Proteinuria, n (%)				
Non-proteinuria	366,824 (90.3)	122,045 (83.2)		
Occasional proteinuria	34,684 (8.5)	21,337 (14.5)	0.212	0
Persistent proteinuria	4,690 (1.2)	3,371 (2.3)		
Lifestyle				
Smoking, n (%)	63,318 (15.9)	19,617 (13.6)	0.066	10,138
Exercise, n (%)*	137,326 (41.6)	48,499 (39.2)	0.050	99,452
Medication				
Anti-diabetic drugs, n (%)	22,306 (5.6)	4,984 (3.4)	0.104	8,829
Anti-hypertensive drugs, n (%)	114,699 (28.7)	38,602 (26.7)	0.046	8,921
Lipid-lowering drugs, n (%)	58,848 (14.7)	23,115 (15.9)	0.034	8,543
Past history				
Stroke, n (%)*	13,290 (3.6)	4,146 (3.1)	0.031	51,887
Heart diseases, n (%)*	21,220 (5.8)	7,499 (5.6)	0.010	51,727
Kidney diseases, n (%)*	2,033 (0.6)	1,058 (0.8)	0.028	50,838

Data are shown as median (interquartile range) or number (percentage) as appropriate.

Significant differences were evaluated by the standardized mean differences (SMD).

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, hemoglobin A1c; eGFR, estimated glomerular filtration rate.

*self-reported habit of exercise, history of stroke, heart diseases and kidney diseases.

Table 2. Baseline characteristic stratified by a proteinuria category.

	Non-proteinuria	Occasional proteinuria	Persistent proteinuria	SMD	Missing, n
Number of subjects	488,869	56,021	8,061		0
Male, n (%)	196,594 (40.2)	30,297 (54.1)	5,522 (68.5)	0.391	0
Age, years	64 (59–68)	65 (60–69)	66 (61–70)	0.185	0
BMI, kg/m^2	22.8 (20.9–24.9)	23.8 (21.6–26.1)	24.9 (22.6–27.4)	0.398	5,637
Observational periods, years	3.0 (1.9–4.1)	3.5 (2.1–4.3)	2.1 (1.1–3.3)	0.453	0
Systolic blood pressure, mmHg	128 (116–139)	132 (120–144)	138 (127–150)	0.394	2,450
Diastolic blood pressure, mmHg	76 (70–82)	80 (70–86)	80 (72–88)	0.258	2,527
Laboratory data					
Triglyceride, mg/dL	100 (72–142)	111 (79–162)	132 (93–194)	0.278	49
HDL cholesterol, mg/dL	61 (51–72)	57 (48–69)	53 (45–64)	0.277	19
LDL cholesterol, mg/dL	125 (105–146)	124 (103–145)	124 (103–146)	0.028	56
HbA1c, %	5.2 (5.0–5.5)	5.3 (5.0–5.7)	5.5 (5.1–6.3)	0.414	12,548
Serum creatinine, mg/dL	0.70 (0.60–0.80)	0.70 (0.60–0.90)	0.90 (0.70–1.10)	0.407	0
eGFR, mL/min/1.73m^2	74.7 (64.7–85.3)	74.1 (63.4–85.0)	64.2 (51.2–75.8)	0.426	0
Uric acid, mg/dL	5.0 (4.2–6.0)	5.4 (4.4–6.4)	6.1 (5.1–7.1)	0.451	14,588
Hematuria, n (%)	122,045 (25.0)	21,337 (38.1)	3,371 (41.8)	0.242	0
Lifestyle					
Smoking, n (%)	69,968 (14.6)	11,019 (20.0)	1,948 (24.4)	0.166	10,138
Exercise, n (%)*	164,089 (40.9)	19,087 (41.6)	2,649 (41.0)	0.010	99,452
Medication					
Anti-diabetic drugs, n (%)	20,386 (4.2)	5,104 (9.3)	1,800 (22.5)	0.375	8,829
Anti-hypertensive drugs, n (%)	126,082 (26.2)	22,392 (40.6)	4,827 (60.4)	0.482	8,921
Lipid-lowering drugs, n (%)	70,195 (14.6)	9,733 (17.7)	2,035 (25.4)	0.182	8,543
Past history					
Stroke, n (%)*	14,423 (3.3)	2,394 (4.7)	619 (8.4)	0.149	51,887
Heart diseases, n (%)*	24,016 (5.4)	3,917 (7.7)	786 (10.7)	0.130	51,727
Kidney diseases, n (%)*	2,106 (0.5)	572 (1.1)	413 (5.6)	0.209	50,838

Data are shown as median (interquartile range) or number (percentage) as appropriate.

Significant differences were evaluated by the standardized mean differences (SMD).

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, hemoglobin A1c; eGFR, estimated glomerular filtration rate.

*self-reported habit of exercise, history of stroke, heart diseases and kidney diseases.

Table 3. The association between baseline hematuria and annual decline rate in eGFR by regression analyses in entire cohort

	Mean annual eGFR decline rate, mL/min/1.73 m^2/year (95% CI)		
	Non-hematuria	Hematuria	Difference
Crude	−0.81 (−0.83 to −0.80)	−0.90 (−0.93 to −0.87)	0.09 (0.05 to 0.12)
Model 1	−0.81 (−0.83 to −0.80)	−0.91 (−0.94 to −0.88)	0.10 (0.06 to 0.13)
Model 2	−0.85 (−0.87 to −0.83)	−0.93 (−0.96 to −0.90)	0.08 (0.04 to 0.11)
Model 3	−0.84 (−0.86 to −0.82)	−0.99 (−1.02 to −0.97)	0.16 (0.12 to 0.19)

Model 1 adjustments: sex and age

Model 2 adjustments: Model 1 plus BMI, systolic blood pressure, anti-hypertensive drugs, anti-diabetic drugs, history of stroke, heart diseases and kidney diseases, and current smoking status.

Model 3 adjustments: Model 2 plus eGFR, HbA1c and uric acid levels

eGFR, estimated glomerular filtration rate; CI, confidence interval; BMI, body mass index; HbA1c, hemoglobin A1c.

Figure Legends

Fig 1. Flowchart of study participants.
eGFR, estimated glomerular filtration rate.

Fig 2. Annual eGFR decline rate stratified with hematuria and proteinuria categories (two-way ANCOVA).
The annual change in eGFR decline estimated by the ordinary least squares method was used for two-way ANCOVA (proteinuria and hematuria categories). Baseline data, including age, sex, BMI, current smoking status, systolic blood pressure, eGFR, HbA1c, uric acid, antihypertensive drugs, antidiabetic drugs, and history of stroke, heart diseases and kidney diseases were used as potential confounders to adjust for covariates in the ANCOVA.
BMI, body mass index; HbA1c, hemoglobin A1c; eGFR, estimated glomerular filtration rate; ANCOVA, analysis of covariance.

Fig 3. The eGFR trajectory with age estimated by Restricted Cubic Spline curve.
Considering the repeat measured eGFR data for each participant, the eGFR trajectory with age was estimated using a restricted cubic spine curve, without adjustment for covariates.
eGFR, estimated glomerular filtration rate.

Fig 4. The eGFR trajectory with age estimated by a multivariate linear mixed effect model.
The eGFR trajectory with age was estimated using a multivariate linear mixed effect model with a random intercept and random slope. The Akaike information criterion was the lowest when both the random intercept and random slope were used in the mixed-effects model. Sex, BMI, current smoking status, systolic blood pressure, eGFR, proteinuria, HbA1c, uric acid, antihypertensive drugs, antidiabetic drugs, and history of stroke, heart diseases and kidney diseases were used to adjust for potential confounders.
BMI, body mass index; HbA1c, hemoglobin A1c; eGFR, estimated glomerular filtration rate.

Fig 5. The eGFR trajectory with age estimated by a multivariate linear mixed effect model in male and female.
After stratified by sex, the eGFR trajectory with age was estimated using a multivariate linear mixed effect model with a random intercept and random slope. BMI, current smoking status, systolic blood pressure, eGFR, proteinuria, HbA1c, uric acid, antihypertensive drugs, antidiabetic drugs, and history of stroke, heart diseases and kidney diseases were used to adjust for potential confounders.
BMI, body mass index; HbA1c, hemoglobin A1c; eGFR, estimated glomerular filtration rate.