

Somatic T-cell Receptor Diversity in a Chronic Kidney Disease Patient Population Linked to Electronic Health Records

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Abstract

Germline and somatic genomic variation represent the bulk of ‘omics data available for precision medicine research. These data, however, may fail to capture the dynamic biological processes that underlie disease development, particularly for chronic diseases of aging such as chronic kidney disease (CKD). To demonstrate the value of additional dynamic precision medicine data, we sequenced somatic T-cell receptor rearrangements, markers of the adaptive immune response, from genomic DNA collected during a clinical encounter from 15 participants with CKD and associated co-morbidities. Participants were consented as part of a larger precision medicine research project at the MetroHealth System, a large urban public hospital in Cleveland, Ohio. Despite the limited sample size, we observed reduced T-cell receptor diversity in relation to biomarkers (creatinine and BUN) of CKD status in this older and mostly African American sample. Overall, these data suggest a relationship between advanced CKD and premature aging of the adaptive immune system and highlight the potential of dynamic ‘omic data to generate novel hypotheses about disease mechanisms and unique opportunities for precision medicine applications.

Keywords: T-cell receptor, T-cell receptor repertoire, immunosequencing, chronic kidney disease, premature aging, electronic health records, African Americans

Introduction

The full vision of precision medicine in the United States is the integration of clinical data with ‘omic data all in the context of lifestyle and the environment [1]. The adoption of electronic health records (EHR) by hospitals, spurred in part by the 2009 American Recovery and Reinvestment Act (ARRA) and the associated Health Information Technology for Economic and Clinical Health (HITECH) Act [2], makes the aggregation and analysis of clinical data, as well as delivery of clinical decisions based on these data, possible. Lifestyle and environmental data collection, while lagging behind clinical data collection, is rapidly evolving with the availability of “wearables” and other portable devices that can upload or stream data automatically into personal health records and eventually EHRs [3].

On the ‘omic front, advances in sequencing and array technology has made the generation of genomic data routine and cost effective in both research settings and clinical settings, the latter of which requires Clinical Laboratory Improvement Amendments (CLIA) certification by the state and the Center for Medicare and Medicaid Services. Currently, more than 28,000 clinical tests offered in the United States are registered in NCBI’s Genetic Testing Registry [4], including 100 described as whole exome or whole genome sequencing. Germline whole exome and whole genome sequencing are routinely ordered in the clinic for the undiagnosed diseases and screening for germline mutations in high risk patients (e.g., *BRCA1* and hereditary breast cancer). Somatic sequencing typically describes tumor sequencing in cancer patients to help determine treatment options and course of disease. Mitochondrial sequencing is also ordered in a clinic setting. While these germline, somatic, and mitochondrial sequencing efforts capture and characterize much of the human (and interacting mitochondrial) genome, they do not capture the dynamic somatic variability of the adaptive immune system.

Adaptive Immune System

The human immune system is broadly divided into the first line of broad defense (innate) and a second line of pathogen-specific defense (adaptive). As its name suggests, the adaptive immune response reacts or adapts to specific pathogens or autoantigens, thereby providing a means for the organism to defend against a diverse and constantly evolving array

of pathogens. The adaptive immune system also prevents future infections by the same pathogen through the production of memory cells.

The adaptive immune response is initiated and regulated by the binding between T-cell receptors (TCRs) and antigens presented by major histocompatibility complex (MHC) class I molecules. TCRs are complexes of alpha/beta chains or delta/gamma chains as well as a cluster of differentiation 3 (CD3) and zeta chains. Most TCRs contain alpha/beta chains, encoded by *TRA* and *TRB* on chromosomes 14 and 7, respectively, and consist of a variable (V) domain, a constant (C) domain, and a joining (J) domain [5, 6]. The beta chain also includes a diversity (D) domain. Germline sequence variation at *TRA* and *TRB* differs by population, with a higher number of variant sites and higher nucleotide diversity estimates in African-descent populations compared with other populations [5, 7, 8].

While germline sequence-level diversity is evident at these loci, the diversity of adaptive immune responses is driven by capability of somatic cells to randomly shuffle the gene segments that make the alpha and beta chains early in TCR development. This somatic shuffling event, or V(D)J recombination, coupled with additional insertion and deletion events, results in novel amino acid sequences in the antigen-binding region of the TCRs (the complementarity determining regions or CDRs) and hence a diverse repertoire of TCRs unconstrained by the static and constant germline sequence.

In healthy individuals, the TCR repertoire is polyclonal with an estimated 10^{13} unique receptor nucleotide sequences [9] or clonotypes [10]. Activation of TCRs occurs when the receptors bind to antigens, resulting in clonal expansion of T cells preparatory to mounting an immune defense against a specific antigen. At the other extreme, monoclonal or oligoclonal T-cell populations may be the result of infectious disease (HIV, Epstein-Barr virus), cancer, autoimmunity, or other diseases that result in immune deficiency (e.g., severe combined immunodeficiency). Within healthy individuals, TCR repertoires also differ by age, with older age associated with decreased diversity [11-15] and an increase in infections and poor response to vaccinations [16]. Recent evidence suggests that TCR repertoires are reduced among individuals with chronic non-autoimmune diseases or conditions such as cardiovascular disease [17], hypertension [18-20], type 2 diabetes [21, 22], and chronic kidney disease (CKD) [23, 24].

Chronic kidney disease and the premature aging phenotype

CKD is common, affecting 26 million adults in the US alone [25]. CKD stage is primarily monitored by measuring kidney function (estimated glomerular function or eGFR) [26] and is categorized as mild (eGFR = 60-89 mL/min; stage 2), moderate (eGFR = 30-59 mL/min; stage 3), severe (eGFR = 15-29 mL/min; stage 4), and end stage (eGFR <15 mL/min; stage 5). End stage renal disease requires dialysis or renal transplant. The prevalence of CKD stages 1-5 in a general adult US population is ~15%, with stages 3 (10.8%) and 5 (1.5%) representing the most and least prevalent CKD stages [27].

Known risk factors for the development of CKD include female sex, increased age, hypertension, and type 2 diabetes [28-30]. CKD risk is also associated with race/ethnicity [28], with African Americans disproportionately representing kidney disease patients as well as those who progress to end stage disease. A proportion of the health disparity can be attributed to the higher rates of hypertension and type 2 diabetes among African Americans compared with other populations. The observed health disparity is also a result of genetic variants within *APOLI* common in African-descent populations and rare or absent in non-African-descent populations [31].

CKD patients develop a premature aging phenotype marked by an increased risk of cardiovascular disease (CVD) [32], osteoporosis, hip fractures, and other conditions associated with aging such as muscle wasting and suppressed immunity [33]. At the cellular level, the CKD premature aging profile is associated with chronic inflammation, decreased T-cell receptor (TCR) activation, and altered TCR diversity profiles [34] including skewed variable(V)beta repertoire [24]. This dynamic premature aging profile among CKD patients differs by sex, race/ethnicity, and chronological age [35-37].

Previous studies of TCR repertoire in CKD patients have been limited to end stage renal disease (ESRD) patients [24] or to patients with primary human cytomegalovirus infection and/or Epstein Barr Virus infection after renal transplantation [38], both representing extreme, less prevalent CKD stage 5. Also, previous studies employed spectratyping assays which, coupled with polyacrylamide gel electrophoresis, result in data that describe the proportion of CDR3 amplicons of each length, informing whether or not the repertoire distribution is skewed (or oligoclonal) [39]. Here we used next-generation sequencing technology to characterize TCR repertoire in 15 patients with CKD stages 2, 3, and 4. In these cross-sectional data, we observed decreased TCR diversity with worsening CKD stage. More broadly, these data demonstrate the potential of adding an additional layer of 'omics to precision medicine research to better understand the impact of a disease state as well as to identify potential risk factors in its development.

Methods

Population

Patients were ascertained from the MetroHealth Medical Center's Division of Nephrology and Hypertension in Cleveland, Ohio. All patients participating in this pilot study provided written, informed consent as well as whole blood for DNA extraction. The MetroHealth Institutional Review Board approved this study.

T-cell receptor beta sequencing and bioinformatics

DNA was extracted on the Qiagen QIAasympy (Hilden, Germany) using standard protocols. For each genomic DNA sample, the CDR3 regions were amplified and barcoded in a two-step multiplex PCR [40] using Adaptive Biotechnologies' immunoSEQ kit per manufacturer's protocol. Amplicons were sequenced with six replicates using Illumina's (San Diego, California) NextSeq sequencing platform. Amplification and sequencing were performed by the University of Miami's Center for Genome Technology. Sequencing data were transferred to Adaptive Biotechnologies (Seattle, Washington) for quality control, alignment, and further processing using their bioinformatics pipeline ANALYZER.

We extracted data and metrics from ANALYZER, including total templates, total productive templates for all productive rearrangements, fraction of productive templates, the number of rearrangements, the number of unique rearrangements, productive clonality, and the maximum productive clonality frequency. Productive rearrangements are defined as the count of unique rearrangements (nucleotide sequence generated through V(D)J recombination) that are in-frame and do not contain a stop codon (i.e., rearrangements that can produce a functional protein receptor). Productive clonality is a Shannon entropy-based measure of clonality for the sample calculated over all productive rearrangements, providing an estimate of both richness (number of unique sequences summarized by entropy, which estimates of the distribution of sequences based on information theoretic measurement of a probability distribution) and evenness (relative abundance of each unique sequence) [41]. Productive clonality ranges from 0 (polyclonal or many rearrangements) to 1 [one (monoclonal) or a few (oligoclonal) predominant rearrangements] and is calculated as $1 - \text{entropy} / \log_2(\# \text{ productive unique rearrangements})$, with entropy accounting for clone frequency.

Electronic phenotyping

We accessed the electronic health records of participating patients from MetroHealth Medical Center in Cleveland, Ohio. The MetroHealth System is a non-profit public healthcare system encompassing 25 locations in the greater Northeast Ohio community of Cuyahoga County. The MetroHealth System installed Epic Electronic for clinical care in 1999, and MyChart, Epic's personal health record, in 2011. In 2014, the MetroHealth System became the first safety-net healthcare system in the United States to achieve the Healthcare Information Management and Systems Society (HIMMS) Stage 7. As of 2015, there were approximately 1.03 million annual outpatient visits and 500,000 covered lives in the EHR.

We extracted billing (ICD-9-CM and ICD-10-CM) codes and laboratory measures recorded in the patients' EHRs from a clinic visit date closest to the time of blood draw. Extracted laboratory measures included those from the basic metabolic panel [creatinine, blood urea nitrogen (BUN), glucose, sodium, calcium, carbon dioxide (bicarbonate), chloride, potassium] and complete blood count [white blood cells (WBC), red blood cells (RBC), platelets (PLT), hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean platelet volume (MPV), and red cell distribution width (RDW_CV)]. We also extracted height, weight, vital signs (temperature, pulse, and respiration rate) as well as systolic and diastolic blood pressures. Estimated glomerular filtration rate was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [26] available from the National Institute of Diabetes and Digestive and Kidney Diseases online calculator (<https://www.niddk.nih.gov/health-information/health-communication-programs/nkdep/lab-evaluation/gfr-calculators/adults-conventional-unit-ckd-epi/Pages/default.aspx>). The CKD-EPI equation requires serum creatinine, sex, age, and race/ethnicity. CKD stages were classified according to eGFR: > 90mL/min (stage 1); 60-89 mL/min (stage 2); 30-59 mL/min (stage 3); 15-29 mL/min (stage 4); and <15 mL/min (stage 5 or end stage).

Statistical analyses

Descriptive statistics (proportions, means, standard deviations, and ranges) were calculated for demographic and clinical variables considered. Statistical differences were assessed using unpaired t-tests, where appropriate. Tests of association were performed as linear regressions between demographic and clinical measures presented in Table 1 (as dependent variables) and productive clonality (independent variable).

Results

Population characteristics

A total of 15 participants ascertained from MetroHealth's Division of Nephrology and Hypertension consented and donated biospecimens for TCRB sequencing. Of the 15 participants, three were European American (20%). More than half of the participants were female (60%), and the average age for all participants at the time of ascertainment was 61.73 years (range: 41-77 years) (Table 1).

Table 1. Study population characteristics

Variable	Mean (\pm SD) or %
Female	60%
Race/ethnicity	
African American	80%
European American	20%
Age (years)	61.73 (10.79)
BMI (kg/m ²)	30.79 (8.54)
Systolic blood pressure (mm Hg)	131.53 (16.92)
Diastolic blood pressure (mm Hg)	71.33 (9.07)
eGFR (mL/min/1.73m ²)	38.07 (17.01)
CKD stage	
2	13.33%
3	53.33%
4	33.33%

As expected, all participants had CKD at the time of ascertainment, with 13.33% at stage 2, 53.33% at stage 3, and 33.33% at stage 4 (Table 1). The distribution of ICD-9-CM and ICD-10-CM billing codes mostly reflected CKD status or its risk factors (hypertension and type 2 diabetes) with the exception of one participant who had ICD-9-CM and ICD-10-CM codes 719.45 and M25.551 for “pain in joint, pelvic region and thigh” and “pain in right hip,” respectively (Table 2). The average body mass index was in the obese range (30.79 kg/m²), and the average systolic and diastolic blood pressures were 131.53 mm Hg and 71.33 mm Hg, respectively, with five participants (33%) in the hypertensive range (systolic blood pressure \geq 140 mm Hg) (Table 1).

We sequenced the T-cell receptor beta CDR3 regions from the genomic DNA of the 15 participants. In this sample, we observed 1,154,792 total templates and an average of 41,508 unique productive rearrangements (Table 3). T-cell receptor diversity, represented by productive clonality, ranged from 0.0151 (Figure 1A) to 0.2565 (Figure 1B) with an average of 0.1030 (Table 3). For comparison, Adaptive Biotechnologies reports a median clonality of ~0.075 for an adult T-cell repertoire in blood (<http://www.adaptivebiotech.com/immunoseq/knowledge-center>).

Consistent with previous reports [24], average productive clonality was lower in females (0.0811) compared with males (1.358), but this difference was not statistically significant ($p=0.125$). Productive clonality was not correlated with age ($R^2 = 0.0045$; $p=0.81$) as might have been expected [14, 15], possibly reflecting both the small sample size and limited age range of this dataset (67% of these patients were born in the 1940s or 1950s).

We then tested for correlations between T-cell receptor diversity and biomarkers of CKD, including disease status calculated using the CKD-EPI equation. Reduced T-cell diversity was observed with increased creatinine ($R^2=0.0995$; $p=0.25$), increased BUN ($R^2=0.0258$; $p=0.57$), and decreased eGFR ($R^2=0.066$; $p=0.36$), but not with white blood cell count ($R^2=0.0004$; $p=0.95$). Reduced T-cell diversity was also noted with worsening CKD status ($R^2=0.2362$; $p=0.07$; Figure 2), with a higher on average productive clonality (0.0488) among stage 4 patients ($n=5$) compared with stage 3 (0.0330; $n=8$) and stage 2 patients (0.0149; $n=2$).

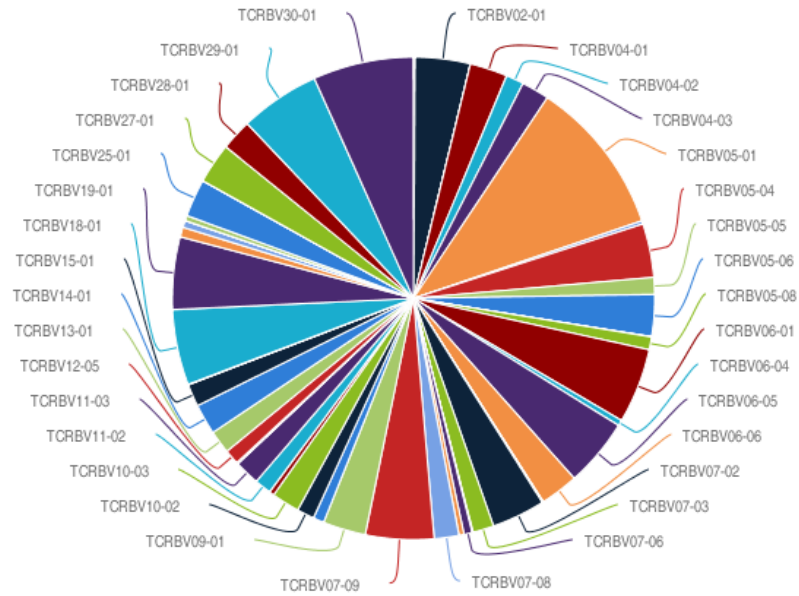
Table 2. Study population ICD-9-CM and ICD-10-CM codes and their descriptions.

ICD-9-CM codes	Description	ICD-10-CM codes	Description
250.4	Diabetes with renal manifestations, type II or unspecified type, not stated as uncontrolled	E11.21	Type 2 diabetes mellitus with diabetic nephropathy
250.42	Diabetes with other manifestations, type II or unspecified type, uncontrolled	E11.22	Type 2 diabetes mellitus with diabetic chronic kidney disease
250.8	Diabetes with other specified manifestations, type II or unspecified type, not state as uncontrolled	E11.29	Type 2 diabetes mellitus with other diabetic kidney complication
401.9	Unspecified essential hypertension	E11.59	Type 2 diabetes mellitus with other circulatory complications
403.90	Hypertensive chronic kidney disease, unspecified, with chronic kidney disease stage I through stage IV, or unspecified	E11.65	Type 2 diabetes mellitus with hyperglycemia
583.81	Nephritis and nephropathy, not specified as acute or chronic, in diseases classified elsewhere	I10	Essential (primary) hypertension
585.3	Moderate with decreased GFR (30-59) Stage III	I12.9	Hypertensive chronic kidney disease with stage 1 through stage 4 chronic kidney disease, or unspecified chronic kidney disease
585.4	Chronic kidney disease, Stage IV (severe)	M25.551	Pain in right hip
585.9	Chronic kidney disease, unspecified	N18.3	Chronic kidney disease, stage 3 (moderate)
719.45	Pain in joint, pelvic region and thigh	N18.4	Chronic kidney disease, stage 4 (severe)
V58.67	Long-term (current) use of insulin	N18.9	Chronic kidney disease, unspecified
		Z79.4	Long-term (current) use of insulin

Conclusion

To our knowledge, we report here the first survey of TCR diversity in CKD patients stages 2-4 using next-generation sequencing technology. Overall, we found that TCR diversity is not strongly associated with age or sex in this sample but reduced TCR diversity was observed with biomarkers of CKD status (creatinine). We also observed a potential relationship between a reduction in TCR diversity and worsening CKD stage. These observations are tempered by the small sample size and limited power, with post-hoc power calculations suggesting that only very large effect sizes ($R^2 > 0.40$) could be detected with 80% power for an association between TCR diversity and the continuous variables (at $p < 0.05$). Nevertheless, these observations are consistent with observations that reduced kidney function results in a uremic milieu that adversely impacts the adaptive immune system [23, 42]. These data further establish the premature aging phenotype of CKD at the cellular level and warrant larger studies to establish its association, if any, with CDK progression.

A.



B.

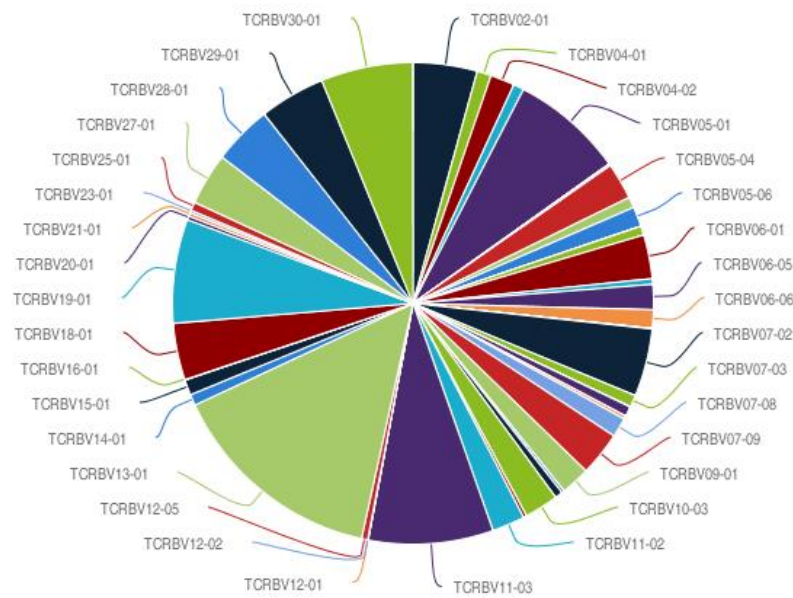


Figure 1. T-cell receptor diversity for two participants. For each participant, V genes detected by sequencing are labeled and color coded, and the pie slices represent the percent of templates represented in each patient's sample. These two participants represent the lowest (most diverse repertoire) (A) and highest (least diverse repertoire) (B) productive clonality values among the 15 participants.

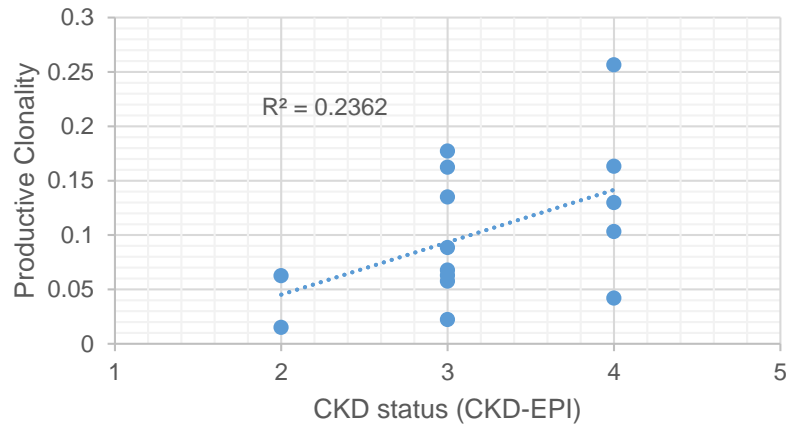


Figure 2. Decreased T-cell receptor diversity and worsening chronic kidney disease status. The x-axis represents CKD stage (CKD-EPI equation) and the y-axis represents the TCR diversity (productive clonality). Productive clonality ranges from 0 to 1 (monoclonal or oligoclonal).

Table 3. Sample-level TCRB sequencing statistics

Variable	Mean (\pm SD) or %	Range
Total templates	76,986 (23,196.93)	35,844 – 115,146
Total productive templates	64,730 (20,541.88)	29,674 – 102,800
Fraction productive	0.8381 (0.03)	0.8013 – 0.8928
Rearrangements	49,782 (17,673.24)	27,826 – 81,617
Productive rearrangements	41,508 (15,044.55)	22,708 – 68,645
Productive clonality	0.1030 (0.07)	0.0151 – 0.2565
Maximum productive frequency	3.59% (0.02%)	0.53% - 10.36%

Our observations in this limited dataset are somewhat consistent with the previous literature. As already noted, the expected association between reduced TCR diversity and increased age was not apparent in this sample set, possibly reflecting both the limited age range and sample size of this study. While we did note that females have higher average TCR diversity compared with males, these differences were not statistically significant. Adaptive immune system sex differences such as higher number of activated T cells and higher counts of specific T cells among females have been noted in some studies [43] but not all [24], and it is unclear if these sex differences impact TCR repertoire. Our data are in agreement with previous reports of altered TCR repertoire in ESRD patients albeit at different resolutions [24].

Next-generation sequencing has heralded a new era of TCR characterization with both research and diagnostic applications. Although these CKD studies are in their infancy, potential precision medicine applications could include TCR sequencing for improved risk prediction, including prognosis for CKD progression and the eventual need for renal replacement therapy or renal transplant. Previous reports suggest that TCR activation is important in developing hypertension [18-20] and type 2 diabetes [21, 22], both major risk factors for the development of CKD, suggesting the disease’s early impact on the adaptive immune system. Periodic TCR clinical sequencing could also identify CKD patients most at risk for infection or could identify a subset of CKD patients who would benefit from altered vaccination schedules or optimized vaccines [44].

This study has several limitations and strengths. The main limitation is sample size. With only 15 patients, we are limited in power to detect statistical differences in TCR repertoire by demographic or clinical variables. Also, this is a cross-sectional study where TCR repertoire is characterized from whole blood at a single point in time. Therefore, longitudinal studies are needed to assess the impact TCR diversity may have on CKD progression and eventual progression to ESRD and its sequelae (e.g., cardiovascular disease). Major strengths of the study include the resolution of TCR repertoire characterization using next-generation sequencing as well as access to the deep clinical data from EHRs. Together, these data suggest an association between advanced CKD and premature aging of the adaptive immune system and highlight the potential of dynamic 'omic data to generate novel hypotheses about disease mechanisms.

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