Supplementary Material – Winkler et al.

Contents

Supplementary Table 1. Sensitivity analyses without log transformation on eGFR for 7 difference locus lead variants.
Shown are DM-status specific meta-analysis results based on eGFR (without log-transformation) including individuals from Uk Biobank and MVP. Results are shown for the seven difference variants.

						DM (UKB + MVP)				noDM (UKB + MVP)				
locusid	**rsid**	**Gene**	**ea**	**oa**	**eaf (UKB,DM)**	**beta**	**se**	**p**	**n**	**beta**	**se**	**p**	**n**	**Pdiff**
d1	rs77924615	*UMOD-PDILT*	G	A	0.80	-2.02	0.11	1.5E-74	77,047	-0.99	0.030	1.3E-241	539,930	**1.4E-19**
d2	rs434215	*TPPP*	A	G	0.27	-0.94	0.10	1.9E-19	77,047	-0.38	0.029	3.5E-41	539,930	**2.8E-07**
d3	rs55722796	*MED1-NEUROD2*	C	T	0.26	7.7E-03	0.17	0.96	19,617	0.52	0.029	4.9E-71	416,964	**3.3E-03**
d4	rs1828678	*CSRNP1*	C	G	0.67	0.35	0.092	1.6E-04	77,047	-0.056	0.025	0.025	539,930	**2.4E-05**
d5	rs963837	*DCDC5*	T	C	0.54	-0.78	0.089	1.8E-18	77,047	-0.47	0.024	2.6E-87	539,930	**6.6E-04**
d6	rs1882963	*NRIP1*	G	C	0.78	0.71	0.11	2.9E-11	77,047	0.24	0.028	2.7E-17	539,930	**2.1E-05**
d7	rs2619264	*SLC22A2*	G	A	0.24	-0.44	0.10	2.7E-05	77,047	-0.16	0.028	4.3E-09	539,930	**0.011**

Supplementary Table 2. GCTA-analysis yields 2 loci with significant secondary joint test signals.

Shown are independent index variants at joint test loci with secondary signals idntified by GCTA based on the EUR-only combined stage results. Abbreviations: bC: conditoned effect size; seC: conditioned standard error; pC: conditioned P value; ea: effect allele; pdiffC: conditioned difference P; pjointC: conditioned joint P

							DM				noDM					
locusid	**signalid**	**SNP**	**SNP_conditioned_on**	**Chr**	**Pos (b37)**	**ea**	**eaf**	**bC**	**seC**	**pC**	**eaf**	**bC**	**seC**	**pC**	**pdiffC**	**pjointC**
js1	1	rs34218958	rs7574806	2	64895903	T	0.81	0.0046	0.0013	4.3E-04	0.80	0.0023	3.3E-04	1.6E-12	0.085	2.9E-14
js1	2	rs7574806	rs7596561[a]	2	64894056	T	0.83	0.0138	0.0032	1.1E-05	0.83	0.0072	0.0017	3.4E-05	0.066	1.2E-08
js16	1	rs7383876	rs12705390	7	105938237	A	0.74	-0.0020	0.0013	0.12	0.74	-0.0020	3.3E-04	7.1E-10	0.98	1.7E-09
js16	2	rs12705390	rs7383876	7	106410777	A	0.21	-0.0054	0.0012	9.0E-06	0.21	-0.0013	3.3E-04	5.5E-05	**0.001**	1.5E-08

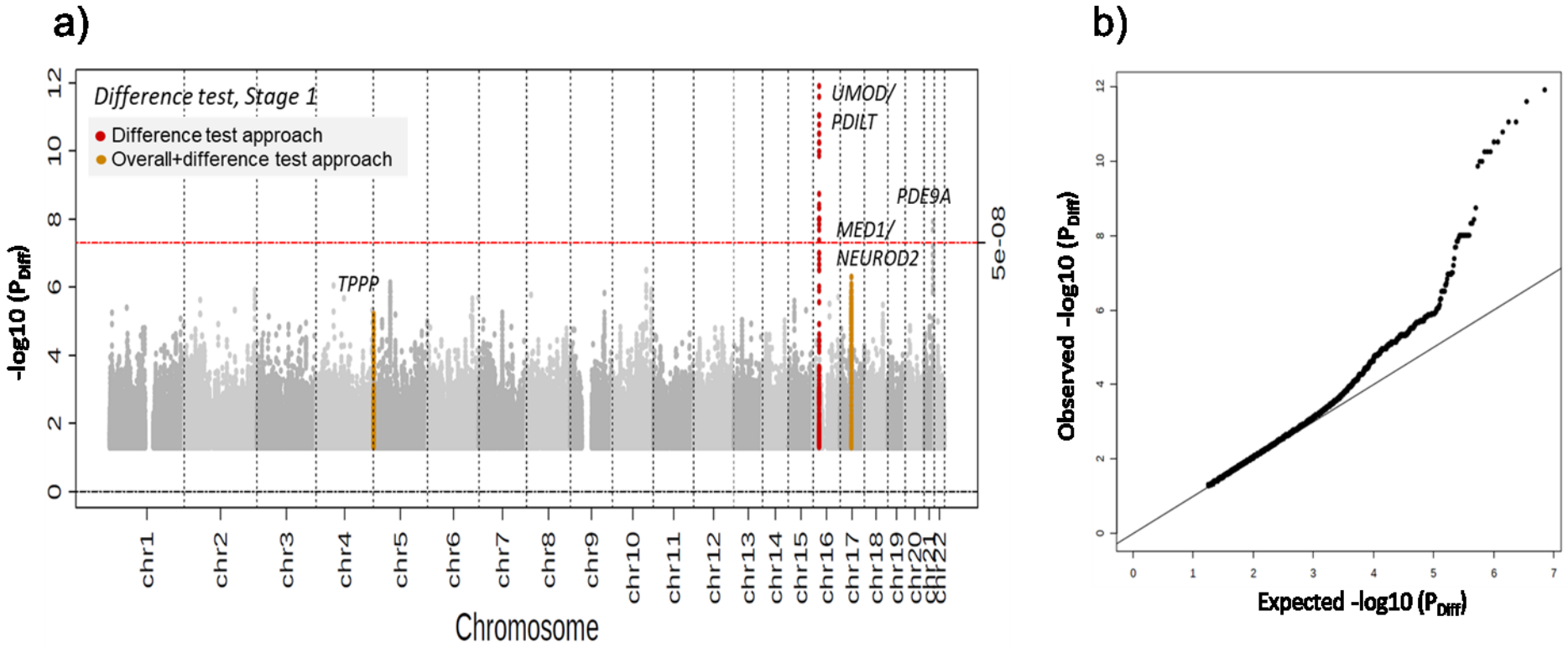
[a] r^2=0.98 to rs34218959

Supplementary Table 3. Human kidney phenotype for genes at 32 novel eGFR loci.

For the candiate genes mapping to the 32 novel eGFRcrea loci, the table shows human kidney-related diseases or phenotypes found in Online Mendelian Inheritance in Man (OMIM) or Groopman et al [1].

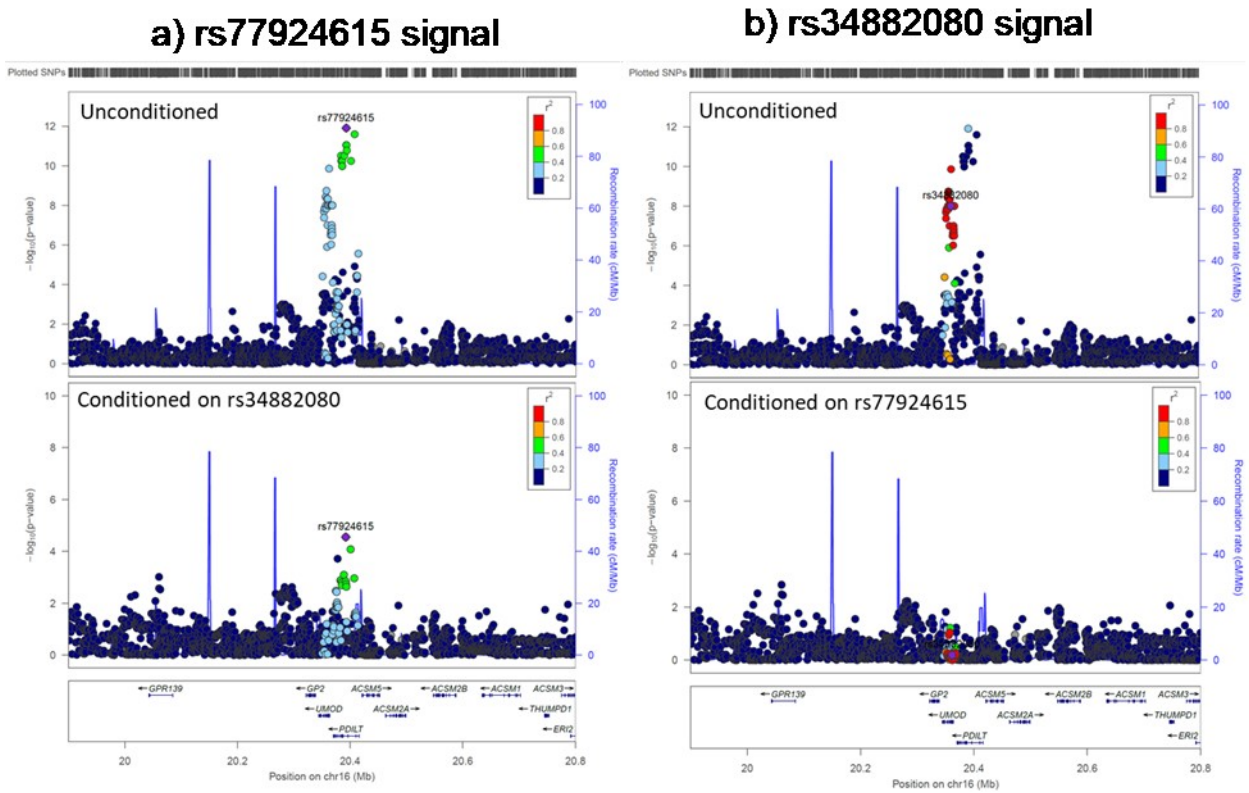
locus_id	Gene	Disease / Phenotype	Source	Chr	Start_of_gene	End_of_gene
js4	*ALPL*	HYPOPHOSPHATASIA, INFANTILE | HYPOPHOSPHATASIA, INFANTILE	OMIM; Groopman et al	1	21835857	21904905
js4	*CDC42*	TAKENOUCHI-KOSAKI SYNDROME; TKS	OMIM	1	22379119	22419436
js4	*HSPG2*	Schwartz-Jampel syndrome	Groopman et al	1	22148724	22263790
js5	*KANK4*	Nephrotic syndrome	Groopman et al	1	62701836	62785083
js9	*SLC2A2*	FANCONI-BICKEL SYNDROME; FBS | FANCONI-BICKEL SYNDROME; FBS	OMIM; Groopman et al	3	170714136	170744768
js11	*SLC30A9*	BIRK-LANDAU-PEREZ SYNDROME; BILAPES	OMIM	4	41992522	42089551
js13	*PPA2*	SUDDEN CARDIAC FAILURE, INFANTILE; SCFI	OMIM	4	106290233	106395227
js18	*EYA1*	OTOFACIOCERVICAL SYNDROME 1; OTFCS | BRANCHIOOTORENAL SYNDROME 1; BOR1	OMIM; Groopman et al	8	72109667	72274467
js19	*CUBN*	Megaloblastic anemia 1-finnish type	Groopman et al	10	16865964	17171816
js20	*RAB18*	Micro syndrome	Groopman et al	10	27793102	27831166
js22	*HPS1*	Hermansky-Pudlak syndrome 1	Groopman et al	10	100175954	100206704
js22	*HPSE2*	UROFACIAL SYNDROME 1; UFS1 | UROFACIAL SYNDROME 1; UFS1	OMIM; Groopman et al	10	100216833	100995632
js24	*C2CD3*	OROFACIODIGITAL SYNDROME XIV; OFD14	OMIM	11	73723758	73882064
js26	*BRCA2*	WILMS TUMOR 1; WT1 | FANCONI ANEMIA, COMPLEMENTATION GROUP D2; FANCD2	OMIM	13	32889616	32973809
js28	*SLC7A7*	LYSINURIC PROTEIN INTOLERANCE; LPI | LYSINURIC PROTEIN INTOLERANCE; LPI	OMIM; Groopman et al	14	23242431	23289020
js31	*FAT4*	HENNEKAM LYMPHANGIECTASIA-LYMPHEDEMA SYNDROME 2; HKLLS2 | VAN MALDERGEM SYNDROME 2; VMLDS2	OMIM; Groopman et al	4	126237566	126414087

Supplementary Figure 1. Three eGFR loci were identified with differential effects by diabetes status in stage 1 and replicated in stage 2
We searched for DM/noDM-differential genetic associations on eGFR using the difference test approach and the overall+difference approach in stage 1 (CKDGen and UKB, n_{DM}=109,993, n_{noDM}=1,070,999). Three difference loci were identified and replicated. a) Shown are difference test P-values over chromosomal base position (Manhattan plot) highlighting the one identified by the difference test approach (red, $P_{Diff}<5x10^{-8}$) and the two loci identified by the overall+difference test approach (orange, $P_{Diff}<8.2x10^{-5}$=0.05/610, corrected for 610 with stage 1 $P_{Overall}<5x10^{-8}$ as published previously [2]). Loci are annotated by the name(s) of the nearest gene(s). b) Shown is the distribution of observed versus expected DM/noDM-difference P-values (QQ plot). The genomic control inflation factor for the stage 1 difference test was λ_{GC}=1.04.



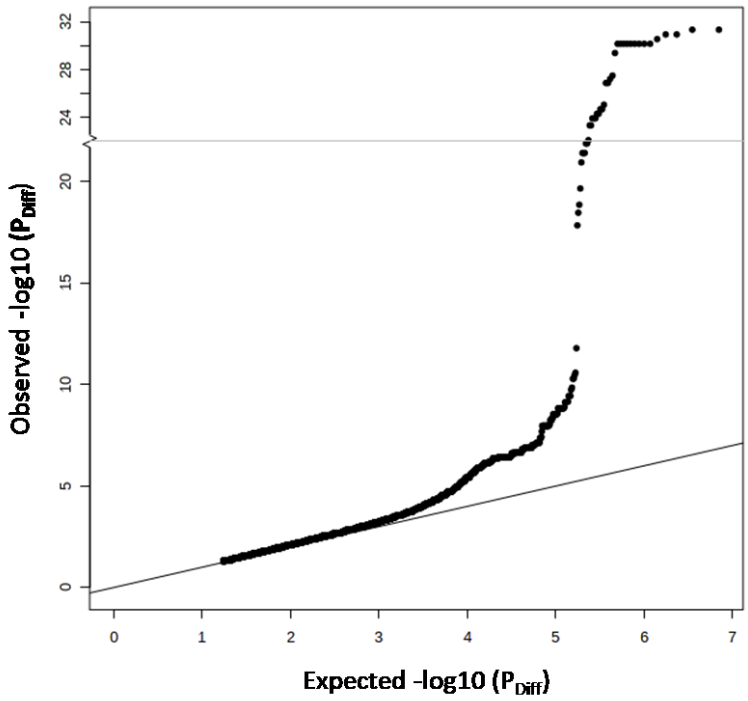
Supplementary Figure 2. Unconditioned and conditioned difference P Values for the *UMOD/PDILT* locus.

We computed difference P-Values at the *UMOD/PDILT* locus unconditioned and conditioned for the two previously identified independent index variants rs77924615 or rs34882080 [3], respectively, in stage 1 (n_{DM}=109,993, n_{noDM}=1,070,999). Shown are difference P-values in regional association plots for each variant in *UMOD-PDILT* locus. a) color-coding correlation to rs79924615 unconditioned (upper panel) and conditioned on rs34882080 (lower panel); and b) color-coding correlation to rs34882080 unconditioned (upper panel) and conditioned on rs79924615 (lower panel). This highlights that the rs77924615 showed a significant difference even after conditioning for rs34882080 but the rs34882080 difference disappeared after conditioning for rs77924615.



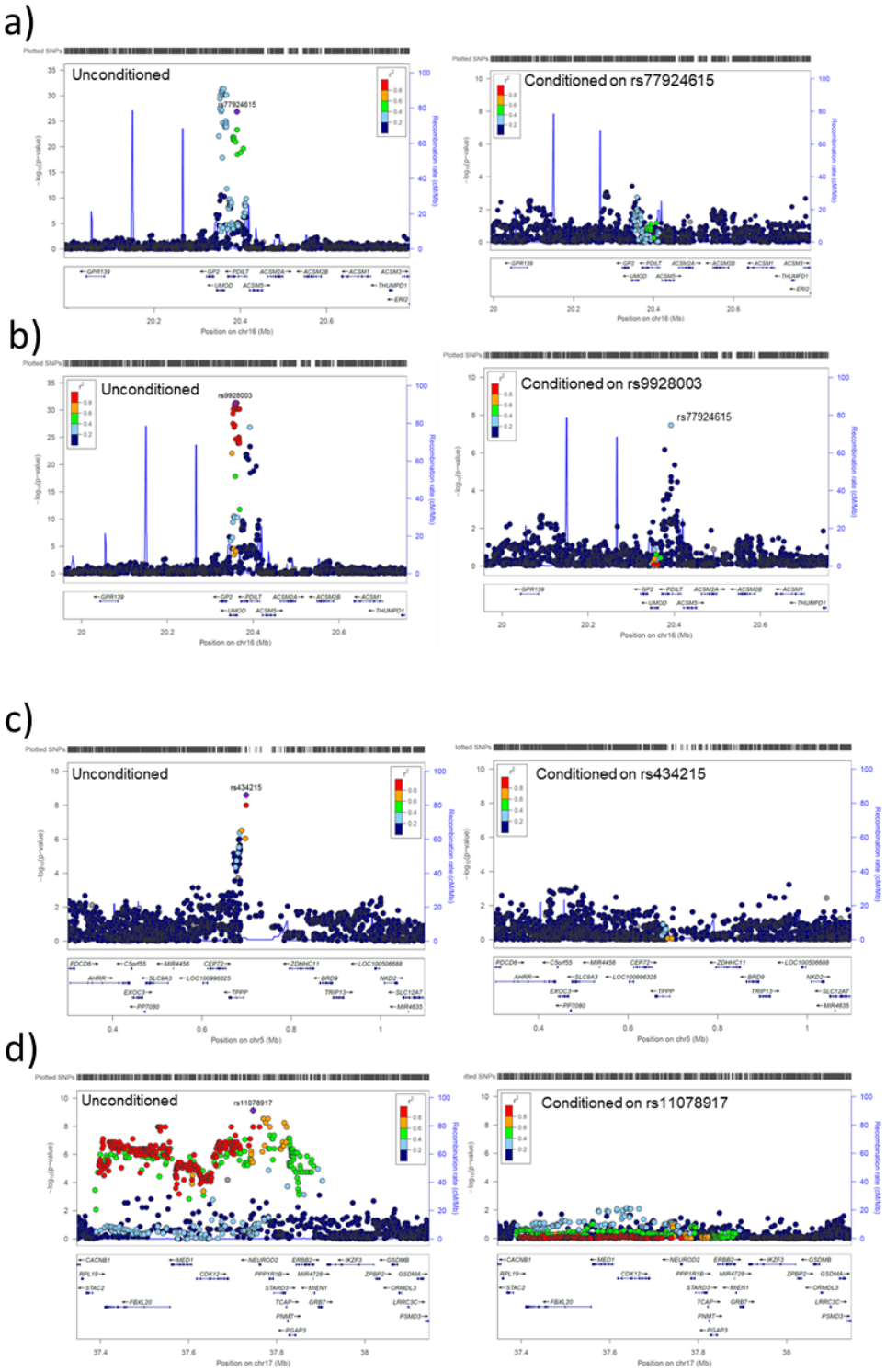
Supplementary Figure 3. Distribution of difference P values in stage 1+2 combined.

Shown is observed difference P-values versus expected (QQ plot) for the combined stages 1+2 (n_{DM}=178,691; n_{nodM}=1,296,113). The genomic control inflation factor for the stage 1+2 difference test was again λ_{GC}=1.04.

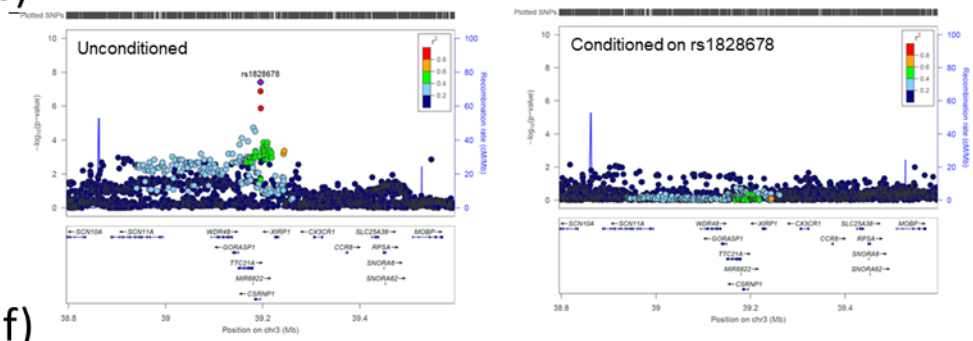


Supplementary Figure 4. Unconditioned and conditioned regional difference P-values for the seven identified difference loci in stage 1+2.

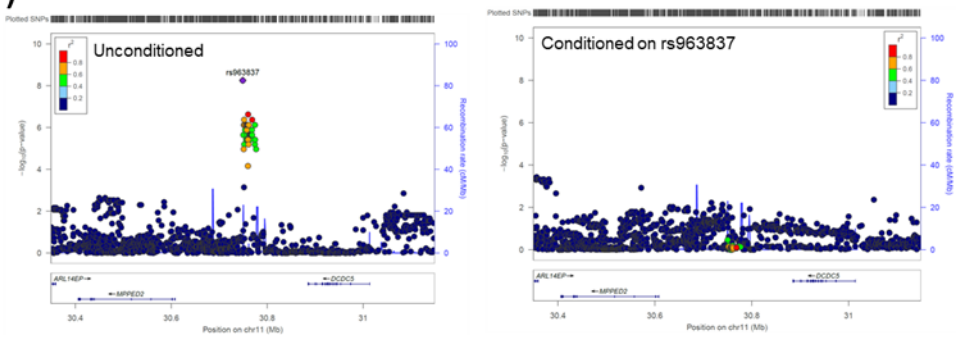
We computed difference P-values for each of the seven identified difference loci unconditioned and conditioned on the respective lead variant based on combined stage 1+2 (n_{DM}=178,691; n_{nodM}=1,296,113). Shown are unconditioned and conditioned difference P-values for the locus near a) *UMOD/PDILT*, conditioned on stage 1 lead variant rs77924615; b) *UMOD/PDILT*, conditioned on stage 1+2 lead variant rs9928003; c) *TPPP*; d) *MED1-NEUROD2*; e) *CSRNP1*; f) *DCDC5*; g) *NRIP1*, and h) *SLC22A2*.



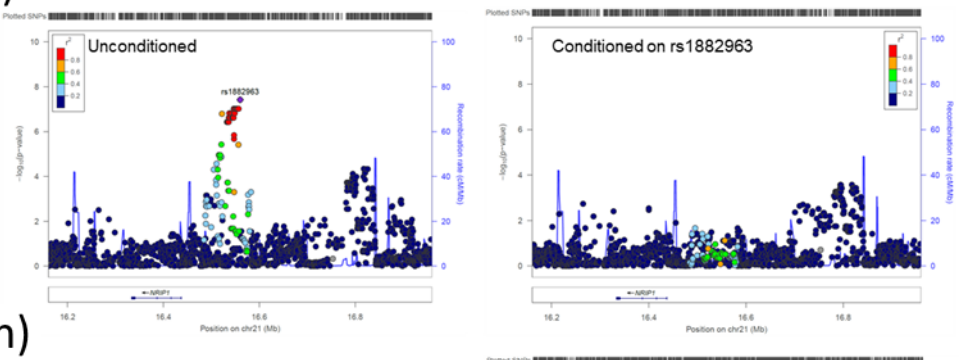
e)


Unconditioned
rs1828678
Conditioned on rs1828678
Position on chr3 (Mb)

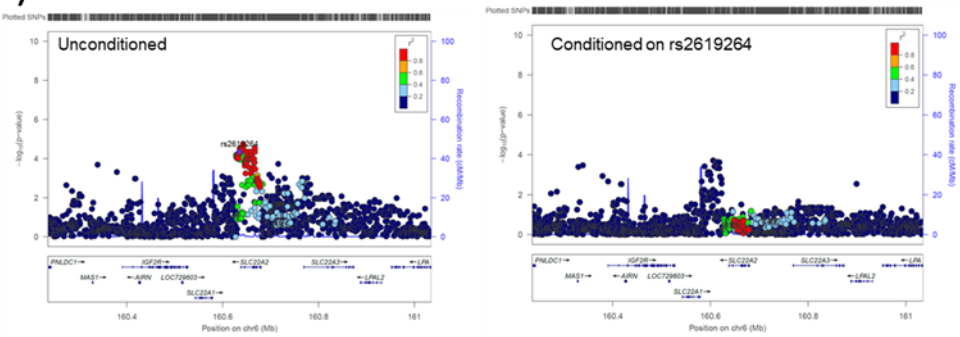
f)


Unconditioned
rs963837
Conditioned on rs963837
Position on chr11 (Mb)

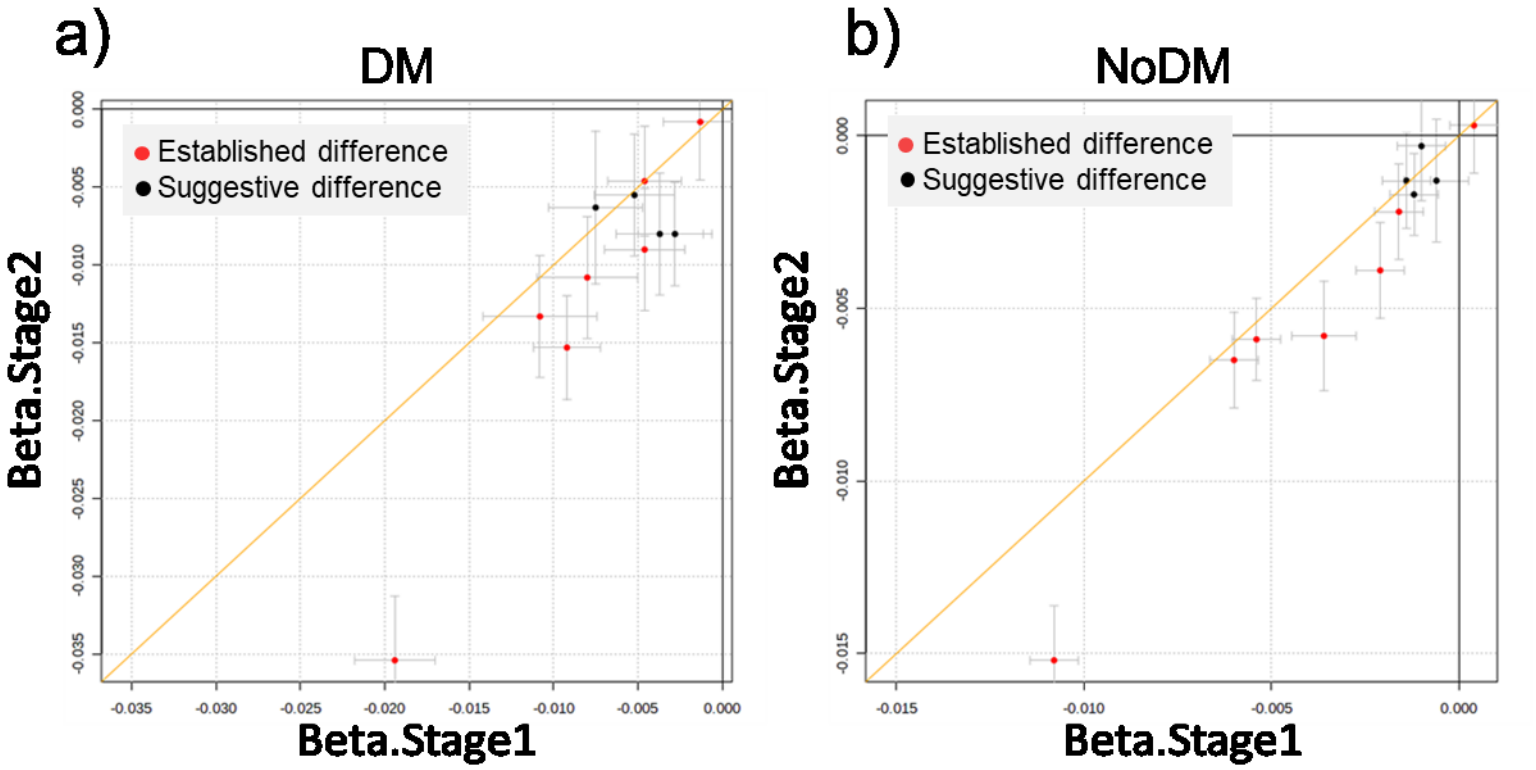
g)


Unconditioned
rs1882963
Conditioned on rs1882963
Position on chr21 (Mb)

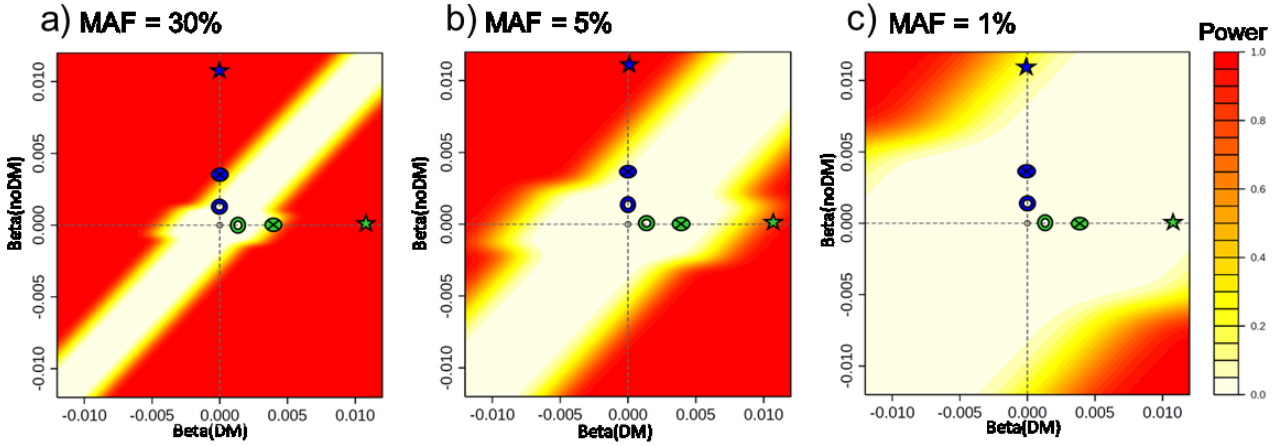
h)


Unconditioned
Conditioned on rs2619264
Position on chr6 (Mb)

Supplementary Figure 5. Stage 1 and stage 2 effect sizes for eGFR by DM and noDM for the 11 differential loci.
Shown are comparisons of effect sizes from stage 1 (n_{DM}=109,993; n_{nodM}=1,070,999) and stage 2 (n_{DM}=68,698, n_{NoDM}=225,114) for a) individuals with DM; or b) individuals with noDM. Indicated in red are the 7 loci identified by the difference screens (from **Table 1**, established difference) and in black the 4 additional novel eGFR loci with suggestive difference (from **Table 2**). Error bars reflect 95% confidence intervals of the estimated genetic effect.



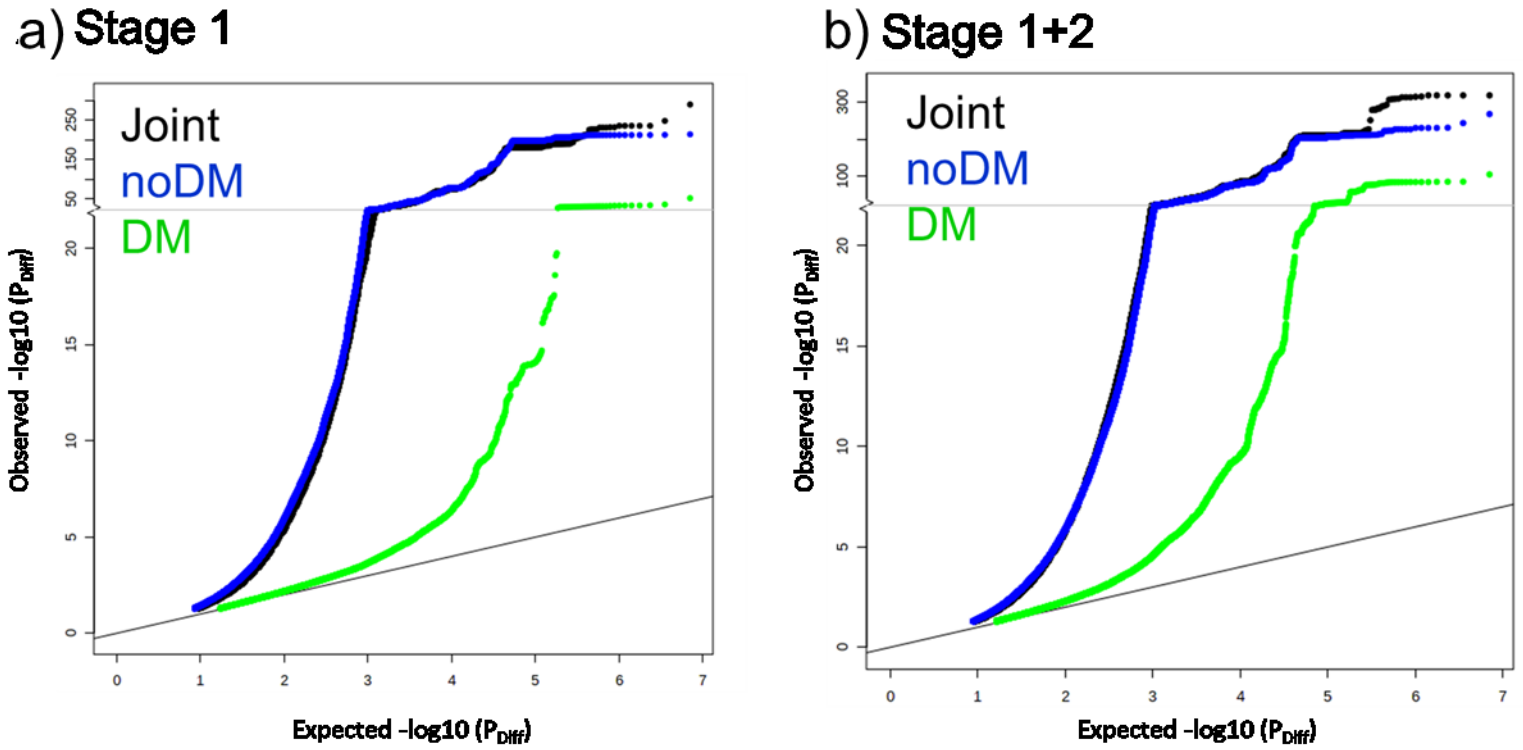
Supplementary Figure 6. Power to detect DM/noDM-differential effects for eGFR for varying effect sizes in DM and noDM.
Based on our stage 1+2 sample size of ~180,000 individuals with DM and ~1,300,000, we computed the power to identify DM/noDM-differential eGFR associations by the difference test approach or the overall+difference test approach for varying log eGFR effect sizes in DM (x-axis) and noDM (y axis). Shown are heatmaps indicating the power (color code) for the respective DM-/noDM effect size for three different minor allele frequencies (MAF). a) common variant (30% minor allele frequency, MAF), b) low frequency variant (5% MAF), and c) rare variant (1% MAF). d) Six scenarios of DM-only or noDM-only effect sizes are highlighted and respective power estimates are given; these scenarios are marked in the heatmaps by different symbols.



d)

	Beta		Power			
Symbol	DM	noDM	AF = 30%	AF = 5%	AF = 1%	Comment
★	0	0.011	>99%	>99%	19%	Large effect in noDM (~*UMOD/PDILT* effect), zero effect in DM
★	0.011	0	>99%	88%	<1%	Large effect in DM (~*UMOD/PDILT* effect), zero effect in noDM
⊗	0	0.004	92%	6%	<1%	Medium effect in noDM, zero effect in DM
⊗	0.004	0	35%	<1%	<1%	Medium effect in DM, zero effect in noDM
○	0	0.001	<1%	<1%	<1%	Small effect in noDM, zero effect in DM
○	0.001	0	<1%	<1%	<1%	Small effect in DM, zero effect in noDM

Supplementary Figure 7. Distribution of joint, DM-only and noDM-only P-values for eGFR.
Shown are the distributions of observed versus expected P-values (QQ plots) from the joint test (black), noDM-only test (blue) and DM-only test (green) for a) stage 1 (n_{DM}=109,993, n_{noDM}=1,070,999) and b) combined stage 1+2 (n_{DM}=178,691; n_{nodM}=1,296,113).



Supplementary Figure 8. Gene priotisation (GPS) at the seven identified difference loci.

By querying the GPS by Stanzick *et al* [2], we identified 7 genes located at 6 of the 7 difference loci that were mapping to a credible variant that was deleteriously protein-relevant within the gene (CADD≥15 [4]), to a credible variant that was an eQTL in kidney tissue [5,6], or genes that were known for kidney-related phenotypes in human (OMIM [7], or Groopman et al. [1]). For presentation in the main manuscript (**Figure 6**), the list of genes with evidenced kidney phenotype was manually reviewed and reduced.

Abbreviations: Position=b37

								99% credible variants within the gene with CADD ≥ 15			99% credible variants within the locus				Evidenced kidney phenotype
											eQTL			sQTL	
							Weights:	1	1	1	1	1	1	1	1
Locus name	Gene	distance to difference variant	Chr of gene	Position start of gene	Position end of gene	credible variants in the signal	Score	stop-gained/ stop-lost/ non-synonymus	canonical-splice/ noncoding-change/ synonymous/ splice-site	other	NEPTUNE glomerulus	NEPTUNE tubulointerstitium	GTEx kidney	GTEx kidney	Human
[UMOD/PDILT]	*UMOD*	-28132	16	20344372	20364200	1	1	0	0	0	0	0	0	0	1
[TPPP]	*SLC6A19*	502663	5	1201709	1225230	2	1	0	0	0	0	0	0	0	1
[TPPP]	*TPPP*	0	5	659976	693510	2	1	0	0	0	0	2	0	0	0
[MED1/NEUROD2]	*PGAP3*	271747	17	37827374	37844323	201	1	0	0	0	0	199	0	0	0
[MED1/NEUROD2]	*CDK12*	62136	17	37617763	37690818	201	1	0	0	1	0	0	0	0	0
[DCDC5]	*PAX6*	1057249	11	31806339	31839509	3	1	0	0	0	0	0	0	0	1
[NRIP1]	*NRIP1*	-122992	21	16333555	16437126	5	1	0	0	0	0	0	0	0	1
[SLC22A2]	*PLG*	487966	6	161123224	161175085	80	2	0	0	0	0	7	0	0	1
[SLC22A2]	*LPA*	317256	6	160952514	161087407	80	1	0	0	0	0	7	0	0	0
[SLC22A2]	*SLC22A1*	-55508	6	160542862	160579750	80	1	1	0	0	0	0	0	0	0
[SLC22A2]	*SLC22A2*	2535	6	160637793	160679963	80	1	0	1	0	0	0	0	0	0

Supplementary Figure 9. Gene prioritization at the 32 novel eGFR loci yields 22 genes.
By querying the GPS derived for the novel eGFR loci according to the approach described previously [2] for the 371 genes at the 32 novel loci (**Supplementary Data 11**), we identified 6 genes mapping to a credible variant with PPA>5% that was deleteriously protein-relevant within the gene (CADD ≥15 [4]), 1 gene mapping to a >5% PPA credible variant that was an eQTL in kidney tissue [5,6], and 15 genes known for kidney-related phenotypes in human (OMIM [7], or Groopman et al. [1]). For presentation in the main manuscript (**Figure 7**), the list of genes with evidenced kidney phenotype was manually reviewed and reduced.

									99% credible variants (PPA > 0.05) within the gene with CADD ≥ 15			99% credible variants (PPA > 0.05) within the locus				Evidenced kidney phenotype for gene
												eQTL			sQTL	
									1	1	1	1	1	1	1	1
Locus name	Gene	distance to signal index variant	Chr of gene	Position start of gene	Position end of gene	credible variants in the signal	credible variants in the signal with PPA> 0.05	Score	stop-gained/ stop-lost/ non-synonymus	canonical-splice/ noncoding-change/ synonymous/ splice-site	other	NEPTUNE glomerulus	NEPTUNE tubulointerstitium	GTEx kidney	GTEx kidney	OMIM Human
[ALPL]	*CDC42*	473515	1	22379119	22419436	40	2	1	0	0	0	0	0	0	0	1
[ALPL]	*ALPL*	-699	1	21835857	21904905	40	2	1	0	0	0	0	0	0	0	2
[ALPL]	*HSPG2*	243120	1	22148724	22263790	40	2	1	0	0	0	0	0	0	0	1
[ARMC4]	*RAB18*	-411251	10	27793102	27831166	201	0	1	0	0	0	0	0	0	0	1
[AUTS2]	*AUTS2*	0	7	69063904	70258054	69	7	1	0	0	1	0	0	0	0	0
[CUBN]	*CUBN*	0	10	16865964	17171816	737	7	2	1	0	0	0	0	0	0	1
[DOCK7]	*KANK4*	-316040	1	62701836	62785083	225	0	1	0	0	0	0	0	0	0	1
[EEF1DP3]	*BRCA2*	390698	13	32889616	32973809	127	2	1	0	0	0	0	0	0	0	2
[FAT4]	*FAT4*	0	4	126237566	126414087	8	8	1	0	0	0	0	0	0	0	2
[LIMCH1]	*SLC30A9*	327736	4	41992522	42089551	62	5	1	0	0	0	0	0	0	0	1
[LOXL4]	*HPS1*	155382	10	100175954	100206704	110	2	1	0	0	0	0	0	0	0	1
[LOXL4]	*HPSE2*	196261	10	100216833	100995632	110	2	1	0	0	0	0	0	0	0	2
[MSC]	*EYA1*	-373623	8	72109667	72274467	11	4	1	0	0	0	0	0	0	0	2
[POLD3]	*C2CD3*	-479550	11	73723758	73882064	72	3	1	0	0	0	0	0	0	0	1
[RASSF6]	*RASSF6*	0	4	74437266	74486348	7	2	1	1	0	0	0	0	0	0	0
[REM2]	*SLC7A7*	-73240	14	23242431	23289020	140	4	1	0	0	0	0	0	0	0	2
[SLC2A2]	*TNIK*	68577	3	170780291	171178197	7	3	1	0	0	0	2	0	0	0	0
[SLC2A2]	*SLC2A2*	2422	3	170714136	170744768	7	3	1	0	0	0	0	0	0	0	2
[SLC2A4]	*DVL2*	-47916	17	7128660	7137863	56	7	1	1	0	0	0	0	0	0	0
[SLC2A4]	*SLC2A4*	0	17	7185053	7191367	56	7	1	0	0	1	0	0	0	0	0
[TET2]	*PPA2*	247206	4	106290233	106395227	48	7	1	0	0	0	0	0	0	0	1
[ZFP36L1]	*ZFP36L1*	-17198	14	69254371	69262960	32	5	1	0	0	1	0	0	0	0	0

Supplementary Note 1. Details on stage 1 overall+difference test approach.

Our stage 1 data (CKDGen and UKB) has been analyzed before for eGFR without DM-/noDM-stratification as described previously [2]. By this, we yielded 634 independent genome-wide significant variant associations ($P_{Overall}<5x10^{-8}$). This was achieved by a step-wise forward selection using approximate conditional analyses: independent variants in a larger region were identified by conditioning on the iteratively identified variants starting by conditioning on the variant with the smallest P-value. Among these 634 variants, 611 variants were analyzable also in DM and noDM, separately. The other 23 variants did not pass quality control filtering thresholds in DM or noDM requiring at least 36 studies in the meta-analysis per stratum. The rs79924715 variant was also excluded because it had already been identified by the difference test approach ($P_{Diff}<5x10^{-8}$). The second variant identified by the difference test approach, rs1223328 near *PDE9A*, had a $P_{Overall}>=5x10^{-8}$ and was thus not among the 634 variants. Taken together, we took 610 variants with $P_{Overall}<5x10^{-8}$ forward to the difference test.

Among the 610 variants, there were three independent variants in the *UMOD-PDILT* locus besides rs79924715, but only one of these, rs34882080, was significant at $P_{Diff}< 0.05/610=8.2x10^{-5}$, **Supplementary Data 2**). When evaluating the independence of the difference association for rs34882080 by conditioning on rs79924715 via GCTA, we found its difference association to disappear, but the rs7992415 difference association to remain (**Supplementary Figure 2**).

Supplementary Note 2. Robustness of the observed differences.

To investigate the robustness of the seven identified DM/noDM-differential eGFR associations, we performed sensitivity analyses using data from UKB and MVP.

First, we evaluated whether the observed patterns of difference were consistent also when eGFR was analyzed on the original scale instead of log-transformed scale [8]. When using combined data from UKB and MVP (n_{DM}=77,047, n_{nodM}=539,930) and eGFR without log-transformation, we observed consistent patterns of differences for all identified variants (**Supplementary Table 1**).

Second, we used a SNP-by-DM interaction term in the regression model (instead of the difference test based on DM-stratified effects sizes in the main analysis) based on linear regression and unrelated individuals of European ancestry in UKB (n_{DM}=19,277, n_{noDM}=348,728). We observed significant interaction effects that were directionally consistent with the difference observed by the stratified approach (**Supplementary Data 4**).

Third, interactions with other correlated covariates unaccounted for in the SNP-by-DM interaction analyses are known potential sources of bias in gene-environment interaction studies [9]. Therefore, we explored extended interaction models using again the unrelated UKB participants of European ancestry (n_{DM}=19,277, n_{noDM}=348,728): the SNP-by-DM interaction

associations were stable also after accounting for SNP-by-Age, SNP-by-Sex, SNP-by-Hypertension or SNP-by-BMI interaction (**Supplementary Data 4**).

In summary, our sensitivity analyses supported the robustness of our observed significant DM/noDM-differential effects at the seven identified loci.

Supplementary Note 3. Power considerations.

We conducted power computations to evaluate the minimum interaction effect size detectable by our combined stage 1+2 data with 180,000 individuals with DM and 1,300,000 individuals with noDM. For this, we considered log-transformed eGFR (age-/sex-adjusted residuals) and a standard deviation as observed in UKB (sd_{DM}=0.21 log(ml/min/1.73m), sd_{noDM}=0.15 log(ml/min/1.73m²)). We calculated power analytically [10] to identify DM/noDM-difference by the difference approach ($P_{Diff}<5x10^{-8}$) or by the overall+difference approach ($P_{Overall}<5x10^{-8}$ and $P_{Diff}<0.05/610$, assuming 610 overall associated variants). We considered different effect sizes on log eGFR in DM and noDM based on realistic effects sizes. In UKB, effect sizes on log eGFR overall of the 424 Stanzick *et al* [2] lead variants ranged from 0.0008 log(ml/min/1.73m²) (small) to 0.013 log(ml/min/1.73m²) (large); the *UMOD/PDILT* lead variant showed a large effect size of 0.011 log(ml/min/1.73m²). The minor allele frequencies (MAF) of these variants ranged from 1% to 50% with a median of 30%.

We first focused on a common variant (MAF=30%) and calculated power for varying log eGFR effect sizes in DM and noDM (**Supplementary Figure 6a**). For example, assuming a large genetic effect on eGFR of 0.011 log(ml/min/1.73m²) (comparable to the *UMOD/PDILT* variant), we had >99% power to identify an interaction when the large effect is noDM- or DM-only (**Supplementary Figure 6a,d**). Considering reduced effect sizes of 0.004 log(ml/min/1.73m²) (medium effect), or 0.001 log(ml/min/1.73m²) (small effect), the power to identify the interaction was (i) 92% and <1%, respectively for a medium and small noDM-only effect and (ii) 35% and <1%, respectively for a medium and small DM-only effect (**Supplementary Figure 6a,d**). For less frequent variants (MAF<=5%), power was limited (**Supplementary Figure 6b-d**).

The minimum interaction effect size detectable at 80% power for a common variant (MAF=30%) was (i) 0.0037 log(ml/min/1.73m²) for a noDM-only effect and (ii) 0.0050 log(ml/min/1.73m²) for a DM-only effect. For low frequency variants (MAF=5%), the minimum detectable effect sizes were (i) 0.0077 log(ml/min/1.73m²) for a noDM-only effect and (ii) 0.011 log(ml/min/1.73m²) for a DM-only effect (**Supplementary Figure 6b,d**).

In summary, the power of the two approaches to detect DM/noDM-differences in genetic effects on eGFR was high for common variants with large noDM-only and DM-only effects as well as for medium noDM-only effects; but limited for medium DM-only effects. For

less frequent or rare variants, realistic DM/noDM-differences in genetic effects on eGFR were not detectable.

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Supplementary Note 6. LifeLines group author genetics.

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