SUPPLEMENTAL MATERIALS

Common Genetic Variants and Peritoneal Solute Transfer Rate in People with Kidney Failure Treated with Peritoneal Dialysis

A Genome Wide Association Study

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**Contributors:** List of key contributors from each participating center

**Supplemental Methods**

***DNA Extraction***

**DNA was purified from either whole blood collected and stored frozen in PAXgene Blood DNA tubes, or from buffy coats. Blood tubes and buffy coats were thawed and red blood cells lysed in buffer containing 150mM NH4Cl, 10mMNaHCO3 and 1.3mM EDTA. The samples were vigorously mixed,** incubated for 10 minutes at room temperature, then centrifuged at 800 x g for 10 minutes. The supernatant was carefully removed. The nuclear pellet was resuspended in 10 ml of Cell Lysis Buffer containing 10mMTris , 10 mM potassium chloride, 10m magnesium chloride, 400mM sodium chloride, 2mM EDTA, and 0.63% SDS. Sample was vortexed at full speed for 20 seconds. Protein was precipitated by addition of 3.3 ml 5M NaCl. The mixture was vortexed at full speed for 20 seconds then centrifuged at 3200 x g for 10 minutes. The supernatant was transferred into a new tube and DNA precipitated with 10 ml isopropanol. The tube was gently rocked until DNA was visible. The DNA was pelleted by centrifugation at 3200 x g for 10 minutes. The pellet was transferred into a 1.5 tube with 1ml of 70% ethanol and spun at full speed in a microcentrifuge. After removal of the supernatant, the pellet was air dried for 10 minutes and resuspended in 500 µl TE. DNA was measured using Quant-iT™ PicoGreen™ dsDNA Kit.

***Approach to winsorization of PD start-PET interval variable***

First, we took low values of PD start-PET interval (< -45d) and scaled these values, then multiplied by a factor of 6 to spread them out, and then took these values minus 45 plus the scaled and factored values to fix the minimum value to -45 while spreading the other values in the tail towards zero with a maximum of -19.2 days. Similarly, with the high values of PD start-PET interval (> 365), we scaled and factored these outliers fixing them in the tail of the distribution with a maximum value possible of 365. We did this in two batches, one batch in the range of 365-1500 days, and a second batch with values > 1500 days. These two batches of outlier high PD start-PET interval values were necessary to tune the spreading factor of the scaled values to not have artificial peaks visible in the histogram of the transformed distribution. For the batch in the range of 365-1500 days we scaled these values, then multiplied by a factor of 60 to spread them out and then took these values plus 365 minus the scaled and factored values to fix the minimum value to 138.38 while spreading the other values in the tail towards zero with a maximum of 365 days. Similarly, for the PD start-PET interval >1500 days we scaled these values, then multiplied by a factor of 30 to spread them out and then took these values plus 365 minus the scaled and factored values to fix the minimum value to 249.2 while spreading the other values in the tail towards zero with a maximum of 365 days.

**Supplemental Table 1.** Number of participants, stratified by country, at each sequential step to be included in the analytic cohort.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Country** | **No. of Centers** | **Enrolled** | **DNA Collected** | **Passed Genotyping Quality Control** | **Phenotype Data Available** | **4-h D/P creatinine 0.30-1.15** | **Genetically Unrelated Participants\*** |
| Australia | 2 | 74 | 72 | 65 | 65 | 65 | 63 |
| Belgium | 3 | 314 | 314 | 264 | 264 | 264 | 258 |
| Canada | 5 | 292 | 266 | 223 | 221 | 221 | 215 |
| Sweden | 1 | 215 | 214 | 192 | 192 | 192 | 190 |
| UK | 43 | 1958 | 1955 | 1693 | 1611 | 1605 | 1575 |
| USA | 15 | 708 | 648 | 573 | 568 | 565 | 549 |
| **TOTAL** | 69 | 3561 | 3469 | 3010 | 2921 | 2912 | 2850 |

\*Analytic Cohort for the GWAS

**Supplemental Table 2:** Number of participants with missing data on covariates, stratified by ancestry

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **European (n=2212)** | **African (n=181)** | **Asian (n=109)** | **Admixed/Other (n=348)** | **Total (n=2850)** |
| Age, n (%) | 3 (< 1%) | 2 (1%) | 2 (2%) | 0 | 7 (< 1%) |
| Diabetes, n (%) | 348 (16%) | 8 (4%) | 4 (4%) | 41 (12%) | 401 (14%) |
| Body Mass Index, n (%) | 55 (3%) | 19 (10%) | 15 (14%) | 25 (7%) | 114 (4%) |
| PD Start-PET Inter, n (%) | 5 (< 1%) | 0 | 0 | 1 (< 1%) | 6 (< 1%) |

**Supplemental Table 3:** Comparison of summary characteristics of covariates in sub-groups with complete data and with each of the five sets of multiple imputations used in the analyses. Standardized mean difference (SMD), SMD = (Meancomplete – Meanimputation)/Std. Dev.complete

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **Complete Data** | **Imputation One** | **Imputation Two** | **Imputation Three** | **Imputation Four** | **Imputation Five** |
| Age, years  n  Median  Mean  Std. Dev.  SMD | 2843  60  58.5  15.8  NA | 2850  60  58.5  15.8  0 | 2850  60  58.6  15.8  -0.006 | 2850  60  58.5  15.8  0 | 2850  60  58.5  15.8  0 | 2850  60  58.5  15.8  0 |
| Diabetes  n  Yes  % | 2449  906  37.0 | 2850  1057  37.1 | 2850  1045  36.7 | 2850  1030  36.1 | 2850  1025  36.0 | 2850  1023  35.9 |
| Body Mass Index, kg/m2  n  Median  Mean  Std. Dev.  SMD | 2736  26.2  27.2  6.0  NA | 2850  26.2  27.3  6.0  -0.016 | 2850  26.2  27.2  6.0  0 | 2850  26.2  27.2  6.0  0 | 2850  26.2  27.2  6.0  0 | 2850  26.2  27.3  6.0  -0.016 |
| Log PD Start-PET Interval  n  Median  Mean  Std. Dev.  SMD | 2813  -0.29  0  1  NA | 2850  -0.21  0  1  0 | 2850  -0.21  0  1  0 | 2850  -0.21  0  1  0 | 2850  -0.21  0  1  0 | 2850  -0.21  0  1  0 |

**Supplemental Table 4**. Comparison of demographic and clinical characteristics of participants excluded with those included in the analyses.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Excluded**  **(N=620)** | **Included**  **(N=2850)** | **Total**  **(N=3470)** | **p value** |
| **COUNTRY, n (%)** |  |  |  | < 0.001 |
| N-Miss | 11 | 0 | 11 |  |
| Australia | 6 (1) | 63 (2) | 69 (2) |  |
| Belgium | 63 (10) | 258 (9) | 321 (9) |  |
| Canada | 29 (5) | 215 (8) | 244 (7) |  |
| Sweden | 25 (4) | 190 (7) | 215 (6) |  |
| UK | 409 (67) | 1575 (55) | 1984 (57) |  |
| USA | 77 (13) | 549 (19) | 626 (18) |  |
| **Sex Female, n (%)** |  |  |  | 0.03 |
| N-Miss | 44 | 0 | 44 |  |
| Female | 247 (43) | 1084 (38) | 1331 (39) |  |
| **Age years** |  |  |  | 0.02 |
| N-Miss | 53 | 7 | 60 |  |
| Mean ± SD | 56.9 ± 17.3 | 58.5 ± 15.8 | 58.3 ± 16.1 |  |
| **Race, n (%)** |  |  |  | < 0.001 |
| White | 479 (77) | 2293 (81) | 2772 (80) |  |
| Black | 29 (5) | 189 (7) | 218 (6) |  |
| Asian | 38 (6) | 194 (7) | 232 (7) |  |
| Native American/Pacific Islander  Pacific Islander | 9 (2) | 29 (1) | 38 (1) |  |
| Not Reported | 52 (8) | 145 (5) | 197 (6) |  |
| Other | 13 (2) | 0 (0) | 13 (0.4) |  |
| **Diabetes, n (%)** |  |  |  | 0.58 |
| N-Miss | 146 | 401 | 547 |  |
| Yes | 169 (36) | 906 (37) | 1075 (37) |  |
| **Cause of Kidney Failure, n (%)** | | |  | < 0.001 |
| Diabetes | 51 (8) | 659 (23) | 710 (21) |  |
| Glomerular Disease | 44 (7) | 578 (20) | 622 (18) |  |
| Hypertension | 26 (4) | 316 (11) | 342 (10) |  |
| Cystic kidney disease | 20 (3) | 258 (9) | 278 (8) |  |
| Other/Unknown | 479 (77) | 1039 (37) | 1518 (44) |  |
| **BMI, kg/m2** |  |  |  | 0.91 |
| N-Miss | 165 | 114 | 279 |  |
| Mean ± SD | 27.3 ± 6.3 | 27.2 ± 6.0 | 27.2 ± 6.0 |  |
| **Dialysate Dextrose for PET, n (%)** | | |  | 0.48 |
| N-Miss | 184 | 0 | 184 |  |
| 2.5% | 333 (77) | 2249 (79) | 2582 (79) |  |
| 1.5% | 31 (7) | 177 (6) | 208 (6) |  |
| 4.25% | 72 (16) | 424 (15) | 496 (15) |  |
| **4hr D/P Creatinine** | | |  | 0.50 |
| N-Miss | 185 | 0 | 185 |  |
| Mean ± SD | 0.71 ± 0.13 | 0.70 ± 0.13 | 0.70 ± 0.13 |  |

**Supplemental Table 5:** Association of single nucleotide variants with suggestive associations identified in the meta-analysis, by ancestry

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **rsID** | **European Ancestry (n=2212)** | | | **African Ancestry (n=181)** | | | **Asian Ancestry (n=109)** | | | **Admixed/Other (n=348)** | | |
| **Estimate**  **(95% CI)** | **SE** | **P** | **Estimate (95% CI)** | **SE** | **P** | **Estimate (95% CI)** | **SE** | **P** | **Estimate (95% CI)** | **SE** | **P** |
| rs76108553 | 0.05  (0.03, 0.07) | 0.011 | 9x10-7 | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| rs111976243 | 0.05  (0.03, 0.07) | 0.011 | 2x10-6 | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| rs2901257 | -0.02  (-0.02, -0.01) | 0.004 | 1x10-5 | -0.02  (-0.05, 0.01) | 0.015 | 0.16 | 0.01  (-0.02, 0.04) | 0.015 | 0.64 | -0.01  (-0.03, 0.004) | 0.009 | 0.11 |
| rs117559199 | 0.04  (0.02, 0.06) | 0.01 | 3x10-5 | NA | NA | NA | NA | NA | NA | 0.06  (0, 0.11) | 0.028 | 0.049 |
| rs28644184 | 0.02  (0.01, 0.02) | 0.004 | 1x10-4 | 0.02  (-0.01, 0.04) | 0.014 | 0.29 | 0.01  (-0.01, 0.04) | 0.013 | 0.35 | 0.02  (0.003, 0.04) | 0.009 | 0.02 |

NA – the association of the SNVs was not tested as the number of individuals with the minor allele in the ancestry group were ≤ 10

**Supplemental Table 6:** Association of single nucleotide variants with suggestive associations identified in the meta-analysis, in sub-groups of individuals of European ancestry from the largest enrolling centers

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **rsID** | **Alleles** | | **Meta-Analyses (n=2850)** | **Belgium (n=258)** | **Sweden (n=190)** |
| **A1** | **A2** | **Estimate** | **Estimate** | **Estimate** |
| rs76108553 | T | C | -0.05  (-0.07, -0.03) | -0.06  (-0.14, 0.03) | -0.10  (-0.17, -0.02) |
| rs2901257 | A | G | 0.02  (0.01, 0.02) | 0.02  (0.04, -0.002) | 0.01  (0.05, -0.02) |
| rs117559199 | A | C | -0.04  (-0.06, -0.03) | -0.10  (-0.17, -0.03) | -0.01  (-0.13, 0.12) |
| rs28644184 | T | C | 0.02  (0.01, 0.02) | 0.01  (-0.01, 0.03) | 0.01  (-0.02, 0.04) |

**Supplemental Table 5: Gene function related biology in the reported suggestive genes**. For the reported genes, alternative gene names are provided and the findings from the literature search are summarized.

| **Gene Name(s)** | **Approved Gene Symbol[HGNC]** | **GeneID [NCBI]** | **Approved gene name (HGNC)** | **Current knowledge about this gene (PMID)** |
| --- | --- | --- | --- | --- |
| ***Suggestive associations in meta-analyses as well as GWAS in participants of European ancestry*** | | | | |
| *LINC01800* | *LINC01800* | 101927438 | long intergenic non-protein coding RNA 1800 | None |
| ***Suggestive associations in meta-analyses only*** | | | | |
| *LINC01561* | *LINC01561* | 404216 | long intergenic non-protein coding RNA 1561 | Involved in breast cancer progression (29890225) |
| *PLPP4; DPPL2; PPAPDC1; PPAPDC1A* | *PLPP4* | 196051 | phospholipid phosphatase 4 | *PLPP4* promotes proliferation and tumorigenesis in lung carcinoma (28851360) |
| *KCNC2; KV3.2* | *KCNC2* | 3747 | potassium voltage-gated channel subfamily C member 2 | *KCNC2* has been associated with childhood obesity and diabetes risk (27623749). A family with deletions of the generegion showed neurodevelopmental delay and ataxia (23475819). |
| *KDM2B; CXXC2; Fbl10; PCCX2; FBXL10; JHDM1B* | *KDM2B* | 84678 | lysine demethylase 2B | *KDM2B* is a epigenetic regulator in several types of cancer (31941533; 29408056; 28506929; 24853546; 31218831; etc.) and involved in epithelial-mesenchymal transition (29772566). |
| ***Suggestive associations in GWAS in participants of European ancestry only*** | | | | |
| *GIMAP6; IAN2; IAN6; IAN-2; IAN-6* | *GIMAP6* | 474344 | GTPase, IMAP family member 6 | *GIMAP6* may have roles in cell survival regulation (NCBI EntrezGene). These include apoptosis (28381553) and phagocytosis (24204963). |
| *LINC01505* | *LINC01505* | 100996590 | long intergenic non-protein coding RNA 1505 | None |
| *PCDH9* | *PCDH9* | 5101 | protocadherin 9 | Downregulation is associated with cellular invasion by myxofibrosarcoma (32523124). It has been shown to be a regulator in many cancers including glioblastoma (29267965), hepatocellular carcinoma (28791409) and ovarian (31632082) as well as other cancers (31059116). |
| 'None' means a PubMed search revealed no articles, or those articles were not related to specific biology possibly related to peritoneal dialysis. | | | | | |

**Supplemental Table 8:** GWAS analyses in the entire cohort, adjusted for principal components for ancestry

| **Genomic Region** | **Variant ID** | **Chromosome** | **Position** | **P value** | **Beta**  **(95% CI)** | **R2\*** | **Minor Allele Frequency, %** | **Minor Allele** | **Major Allele** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *LINC01800 5’40kb* | rs76108553 | 2 | 65039700 | 4.1 x 10-6 | -0.05  (-0.07, -0.03) | 0.996 | 2.6 | T | C |
| *LINC01561, PLPP 3’ 5kb* | rs2901257 | 10 | 122358463 | 6.2 x 10-7 | -0.02  (-0.02, -0.01) | 0.998 | 47.4 | G | A |
| *KCNC2 5’ 20kb* | rs117559199 | 12 | 75424551 | 4.0 x 10-6 | -0.04  (-0.06, -0.03) | 0.874 | 3.4 | A | C |
| *KDM2B intron* | rs28644184 | 12 | 121961947 | 4.0 x 10-7 | 0.02  (0.01, 0.02 | 0.999 | 43.6 | T | C |
| *BAZ1A intron* | rs10146956 | 14 | 35267326 | 2.6 x 10-6 | 0.05  (0.03, 0.07) | 0.994 | 2.8 | A | G |
| *LOC284240 ncRNA* | rs515754 | 18 | 77346682 | 4.7 x 10-6 | 0.02  (0.01, 0.02) | 0.998 | 49.2 | G | A |

**Supplemental Figure 1:** **Identity by descent (IBD) plot of all pair-wise comparisons of genetic relatedness.** IBD0 is the probability that the two pairwise individuals represented by each point share zero alleles by descent. IBD1 is the probability that the two pairwise individiauls are represented by each point share one allele by descent. The points near the origin (0,0) are twins and/or sample duplicates and were removed from analysis. The points near (0,1) are parent-child pairs and the points near (0.2, 0.5) are siblings.

Chart, scatter chart

Description automatically generated

**Supplemental Figure 2:** Principal component analysis (PCA) of the autosomal variants to determine genetic ancestry. The plotted points represent the first two principal components (PC1 and PC2) that explain genomic variability in the population. The point label colors are based on the self-reported ancestry as indicated in the figure legend.

**Chart, scatter chart

Description automatically generated**

**Supplemental Figure 3:** QQ plot of GWAS in participants of European ancestry, adjusted for principal components of ancestry in the group using the GCTA software with the -mlma-loco option.

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**Supplemental Figure 4 (page 1 of 2):** Regional association plot of 6 genomic regions with single nucleotide variants with suggestive associations in the meta-analyses or in GWAS of participants of European ancestry (panels A-F).

(A) rs76108553 in *LINC01800* region on chromosome 2 (meta-analyses and in GWAS in participants of European ancestry); (B) rs2901257 near *PLPP4* and *LINC01561* on chromosome 10 (meta-analyses only); (C) rs117559199 near *KCNC2* on chromosome 12 (meta-analyses only); and (D) rs73474862 in *GIMAP6* intron on chromosome 7 (in GWAS in participants of European ancestry only).

**A B**

Chart, scatter chart

Description automatically generatedA picture containing chart

Description automatically generated

C D

Chart, scatter chart

Description automatically generatedGraphical user interface, chart, scatter chart

Description automatically generated

**Supplemental Figure 4 (page 2 of 2):** Regional association plot of 6 genomic regions with single nucleotide variants with suggestive associations in the meta-analyses or in GWAS of participants of European ancestry (panels A-F).

(E) rs11789496 in *LINC01505* intron on chromosome 9 (in GWAS in participants of European ancestry only); and (F) rs7337043 in *PCDH9* region on chromosome 13 (in GWAS in participants of European ancestry only).

**E F**

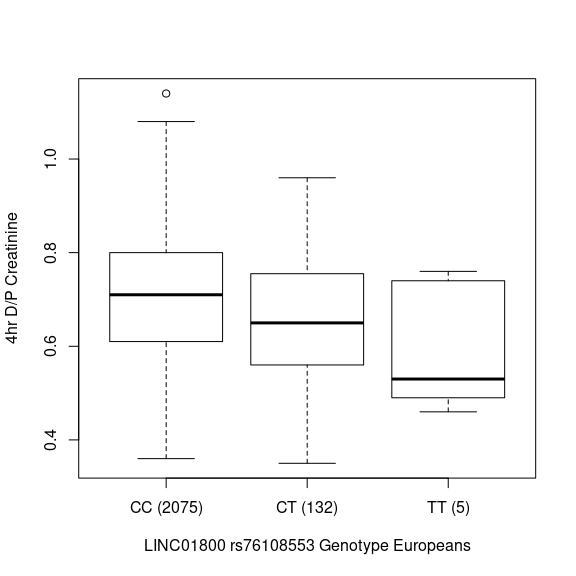
Chart, scatter chart

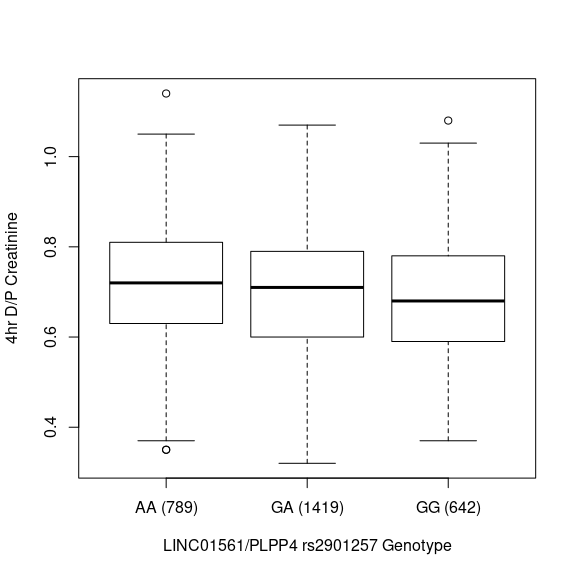
Description automatically generatedChart, scatter chart

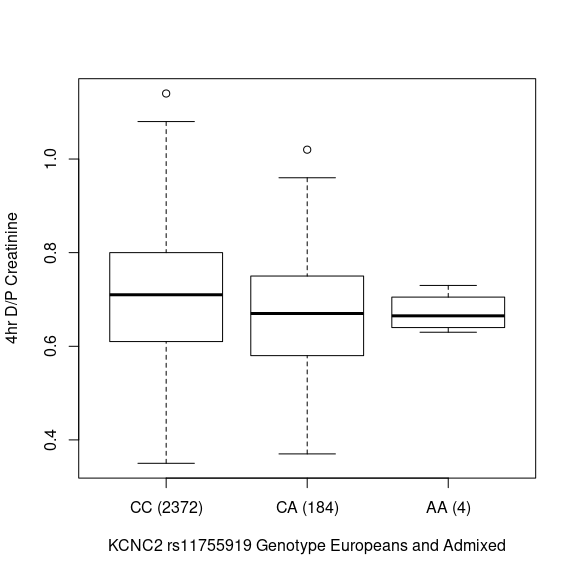
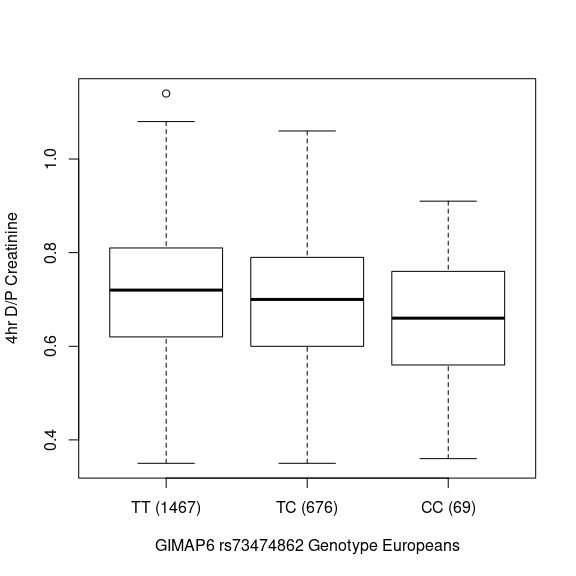
Description automatically generated

**Supplemental Figure 5 (page 1 of 2):** Box plots for PSTR by genotype of variants identified to have suggestive associations in either the meta-analysis or in GWAS in participants of European ancestry (panels A-F).

(A) rs76108553 in *LINC01800* region on chromosome 2 (meta-analyses and in GWAS in participants of European ancestry); (B) rs2901257 near *PLPP4* and *LINC01561* on chromosome 10 (meta-analyses only); (C) rs117559199 near *KCNC2* on chromosome 12 (meta-analyses only); and (D) rs73474862 in *GIMAP6* intron on chromosome 7 (participants of European ancestry ).

**A. B.**

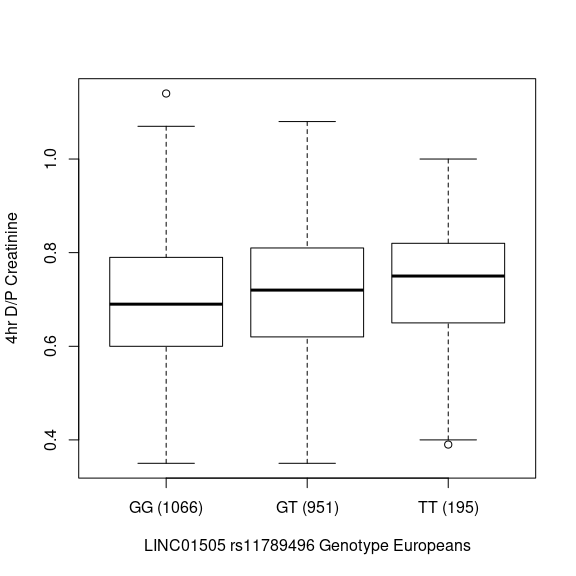
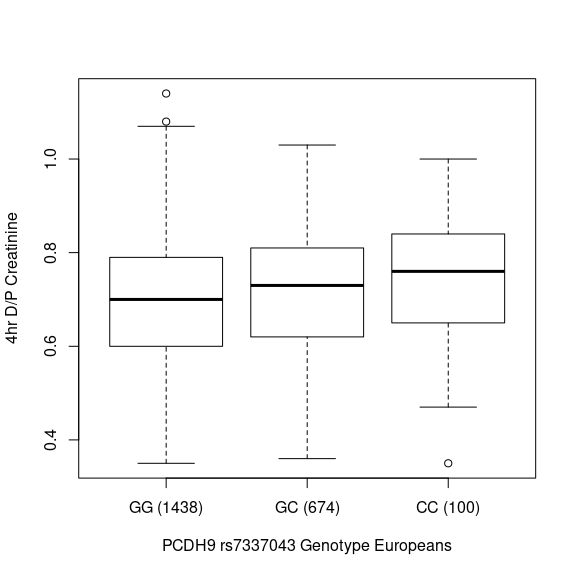


**C. D.**

**Supplemental Figure 5 (page 2 of 2):** Box plots for PSTR by genotype of variants identified to have suggestive associations in either the meta-analysis or in GWAS in participants of European ancestry (panels A-F).

(E) rs11789496 in *LINC01505* intron on chromosome 9 (in participants of European ancestry); and (F) rs7337043 in *PCDH9* region on chromosome 13 (in participants of European ancestry).

**E. F.**



**Supplemental Figure 6:** Results of genome wide association study (GWAS) in the entire cohort, adjusted for principal components of ancestry as - log p plot (Manhattan plot).

Chart, bar chart

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