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## ABSTRACT

Phosphorus is an essential mineral that maintains cellular energy and mineralizes the skeleton. Because complex actions of ion transporters and regulatory hormones regulate serum phosphorus concentrations, genetic variation may determine interindividual variation in phosphorus metabolism. Here, we report a comprehensive genome-wide association study of serum phosphorus concentration. We evaluated 16,264 participants of European ancestry from the Cardiovascular Heath Study, Atherosclerosis Risk in Communities Study, Framingham Offspring Study, and the Rotterdam Study. We excluded participants with an estimated GFR $<45$ ml/min per 1.73 $m^2$ to focus on phosphorus metabolism under normal conditions. We imputed genotypes to approximately 2.5 million single-nucleotide polymorphisms in the HapMap and combined study-specific findings using meta-analysis. We tested top polymorphisms from discovery cohorts in a 5444-person replication sample. Polymorphisms in seven loci with minor allele frequencies 0.08 to 0.49 associate with serum phosphorus concentration ($P = 3.5 \times 10^{-16}$ to $3.6 \times 10^{-7}$). Three loci were near genes encoding the kidney-specific type IIa sodium phosphate co-transporter (*SLC34A1*), the calcium-sensing receptor (*CASR*), and fibroblast growth factor 23 (*FGF23*), proteins that contribute to phosphorus metabolism. We also identified genes encoding phosphatases, kinases, and phosphodiesterases that have yet-undetermined roles in phosphorus homeostasis. In the replication sample, five of seven top polymorphisms associate with serum phosphorous concentrations ($P < 0.05$ for each). In conclusion, common genetic variants associate with serum phosphorus in the general population. Further study of the loci identified in this study may help elucidate mechanisms of phosphorus regulation.

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Phosphorus is an essential mineral that is responsible for maintaining cellular energy, mineralizing the skeleton, and protecting nonosseus tissue from calcification.[1] In humans, the majority of phosphorus resides within bone and teeth as hydroxyapatite and within cells as a component of nucleic acids and phospholipid membranes. A small proportion of phosphorus (approximately 1%) circulates in the serum under tight regulation by the complex actions of specialized ion transporters and regulatory hormones, which balance gastrointestinal phosphorus absorption, bone uptake, cellular flux, and excretion through the kidneys.[2]

Dysregulated phosphorus metabolism may lead to adverse clinical consequences. In the setting of chronic kidney disease, higher serum phosphorus concentrations are associated with vascular calcification and mortality.[3,4] Higher serum phosphorus concentrations within the normal laboratory range are also associated with incident cardiovascular events among individuals without known kidney disease.[5,6] In cell culture models, phosphorus directly transforms vascular smooth muscle tissue into osteoblast-like cells that calcify the medial vessel wall.[7,8]

Several lines of evidence suggest that interindividual differences in the steady-state serum phosphorus concentration may be partly heritable. Rare phosphorus wasting disorders, such as hypophosphatemic rickets, are caused by specific mutations in genes within phosphorus metabolic pathways.[9,10] Genetic disruption of hormones that regulate phosphorus alters circulating phosphorus concentrations in animal models.[11,12] Moreover, dietary phosphorus intake is only weakly associated with the serum phosphorus concentration in humans.[13]

We conducted the first large-scale genome-wide association study (GWAS) to investigate common genetic variants associated with serum phosphorus concentrations in the general population. We conducted a meta-analysis of results from 16,264 individuals of European ancestry participating in the Cardiovascular Heath Study, the Atherosclerosis Risk in Communities Study, the Framingham Heart Study, and the Rotterdam Study.

## RESULTS

### Genome-wide Associations for the Serum Phosphorus Concentration

A total of 16,264 study participants from four cohorts were available for meta-analysis (Table 1). Seven genetic loci located on chromosomes 1, 3, 5, 6, and 12 met the prespecified meta-analysis statistical significance threshold for the association with serum phosphorus (Figure 1). The strongest statistical association was observed for locus 1p36.13 ($P = 3.5 \times 10^{-16}$). The top single-nucleotide polymorphism (SNP) within this locus (rs1697421) does not reside within any known gene, but lies within 10 kb of genes encoding for tissue-nonspecific alkaline phosphatase (*ALPL*) and neuroblastoma breakpoint family member 3 (*NBPF3*; Table 2, Figure 2A).

On chromosome 3, the top SNP within locus 3q21.1 (rs17265703) was in strong linkage disequilibrium ($R^2 = 0.93$) with a second SNP (rs1801725), which represents a known alanine (A) to serine (S) polymorphism of amino acid 986 in the calcium-sensing receptor (*CASR*) gene (Figure 2B).[14]

On chromosome 5, locus 5q35.3 contained several significant SNPs that were associated with serum phosphorus (Figure 2C). The top SNP was located within an intron of *RGS14*, a regulator of G-protein signaling that lies directly adjacent to *SLC34A1*, which encodes for the kidney-specific type IIa sodium phosphate co-transporter (Npt2a).

Three distinct loci on chromosome 6 (6p21.31, 6q23.2, and 6q23.1) were each associated with differences in the circulating serum phosphorus concentration (Figure 2, D through F). Loci on 6q23.1 and 6q23.2 are presumed independent because they are >4000 kb apart and are not correlated ($R^2 < 0.001$), and simultaneous adjustment for both SNPs did not alter the results for either. Locus 6p21.31 contains two intronic SNPs (rs6942022 and rs9469578) in strong linkage disequilibrium within the inositol hexakisphosphate kinase 3 (*IHPK3*) gene. The top significant SNP at locus 6q23.2 is located near the gene encoding for phosphodiesterase 7B (*PDE7B*). The top significant SNP at locus 6q23.1 is located within an intron of the ectonucleotide pyrophosphatase/phosphodiesterase 3 (*ENPP3*) gene, which lies adjacent to the *ENPP1* gene.

On chromosome 12, locus 12p13.32 lies within an open reading frame that is adjacent to RAD51-associated protein (*RAD51AP1*) and fibroblast growth factor 6 (*FGF6*). The gene encoding for the phosphaturic factor fibroblast growth factor 23 (FGF23 ) is located 133 kb upstream from this top SNP; however, there is a recombination point between them (Figure 2G). Several SNPs within the *FGF23* gene were close to meeting genome-wide statistical significance for the association with serum phosphorus concentration.

### Replication

There were 5444 individuals available for the replication meta-analysis (Table 3). For all seven SNPs measured in replication, regression coefficients were in the same direction as those from the discovery sample. On the basis of $\beta$ coefficients adjusted for bias in the discovery cohort and minor allele frequencies for the top seven SNPs, statistical power for replication was limited in some cases. For five of the top SNPs, replication associations were significant at the $P = 0.05$ level, and three were associated at the Bonferroni-corrected $P = 0.007$ level (Table 3).

### Interactions by Sex and Estimated Kidney Function

Associations of the seven top SNPs with the serum phosphorus concentration were similar in magnitude comparing men with women, and no significant interactions were found (all $P$ for interaction >0.1). Results also remained similar after restricting the analysis to individuals who had an estimated GFR (eGFR) ≥60 ml/min per 1.73 $m^2$.

### Gene Score Model

A gene score model that encompassed the sum of risk alleles from the top seven SNPs was significantly associated with the

**Table 1.** Characteristics of the discovery and replication cohorts

| | Discovery Cohorts | | | | Replication Cohorts | | | |
|---|---|---|---|---|---|---|---|---|
| | CHS ($n$ = 1761) | FHS ($n$ = 2865) | ARIC ($n$ = 8048) | RS ($n$ = 3516) | KORA-F3 ($n$ = 1599) | KORA-F4 ($n$ = 1779) | Health ABC ($n$ = 1507) | Vis ($n$ = 659) |
| Age, years | 71.0 | 43.6 | 54.3 | 69.4 | 62.5 | 60.9 | 73.7 | 56.8 |
| | (4.6) | (9.8) | (5.7) | (9.1) | (10.1) | (8.9) | (2.8) | (15.3) |
| Women | 69.6% | 52.1% | 52.9% | 59.4% | 50.5% | 51.3% | 46.9% | 60.1% |
| eGFR, ml/min | 82.3 | 103.4 | 89.7 | 80.7 | 80.1 | 81.9 | 72.4 | 95.7 |
| | (21.4) | (35.9) | (17.7) | (14.9) | (18.1) | (18.0) | (13.4) | (34.7) |
| eGFR 45 to 60 ml/min | 14.3% | 3.2% | 2.7% | 4.8% | 11.5% | 7.6% | 21.5% | 10.2% |
| Serum phosphorus | | | | | | | | |
| Mean, mg/dl | 3.60 | 3.15 | 3.41 | 3.68 | 3.30 | 3.39 | 3.51 | 3.50 |
| (SD) | (0.49) | (0.44) | (0.48) | (0.60) | (0.55) | (0.50) | (0.48) | (0.54) |
| Median, mg/dl | 3.6 | 3.2 | 3.4 | 3.7 | 3.3 | 3.4 | 3.5 | 3.5 |
| (IQR) | (3.3, 3.9) | (2.9, 3.4) | (3.1, 3.7) | (3.3, 4.1) | (2.9, 3.7) | (3.1, 3.7) | (3.2,3.8) | (3.1, 3.9) |

CHS, Cardiovascular Health Study; FHS, Framingham Health Study; ARIC, Atherosclerosis Risk in Communities Study; RS, Rotterdam Study; eGFR, estimated glomerular filtration rate; IQR, interquartile range.

serum phosphorus concentration in the discovery sample ($P = 2.6 \times 10^{-57}$; unadjusted for regression to the mean). The majority of individuals had gene scores between 3 and 12 (Figure 3). In aggregate, the top seven SNPs identified in this study explained approximately 1.5% of the variation in serum phosphorus concentrations (model $R2 = 0.0147$). Serum phosphorus concentrations ranged from 3.24 to 3.58 mg/dl across the spectrum of risk allele dosage.

## DISCUSSION

In this first genome-wide association study of the serum phosphorus concentration, we identified seven genetic loci, located on chromosomes 1, 3, 5, 6, and 12, that were associated with the circulating serum phosphorus concentration. In replication meta-analysis, five of these loci were associated with serum phosphorus at the $P = 0.05$ level and three were associated at the $P = 0.007$ level. Three loci identified in the discovery analysis were near genes that encode for proteins known to be involved in phosphorus metabolism: the sodium phosphate co-transporter type IIa, the calcium-sensing receptor, and *FGF23*. Other candidate genes identified in this study include a phosphatase, a kinase, and two phosphodiesterases that have a yet-undetermined role in the regulation of phosphorus balance.

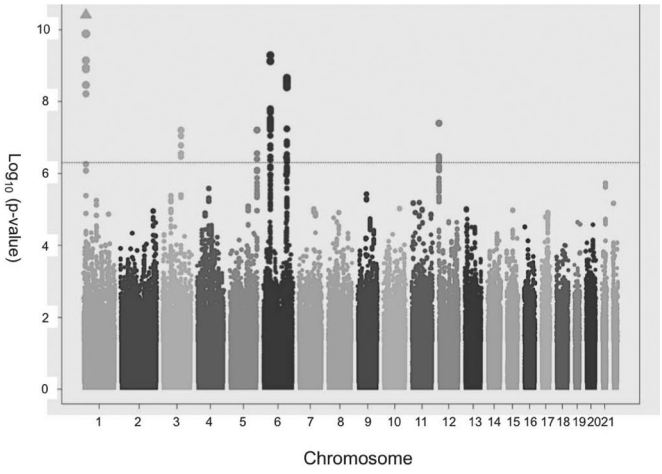


**Figure 1.** Single nucleotide polymorphisms on chromosomes 1, 3, 5, 6, and 12 associate with the serum phosphorus concentration. Log (*P* values) for individual SNPs plotted for each chromosome in the discovery sample. Horizontal line represents genome-wide significance level of $4 \times 10^{-7}$.

Some of the genes identified in this study support previously understood biology of phosphorus metabolism. The sodium phosphate co-transporter type IIa (Npt2a) is expressed in the proximal tubule of the kidney and plays a major role in determining urinary phosphorus excretion.[2] Serum phosphorus excess leads to the removal of Npt2a transporters from the cell-surface, resulting in diminished phosphorus re-absorption and subsequent elimination of phosphorus in the urine.[15] Conversely, a low serum phosphorus concentration stimulates upregulation of Npt2a, which enhances phosphorus re-absorption in the kidney. Deletion of the Npt2a gene in animal models leads to marked urinary phosphorus wasting and impaired skeletal development.[11] Heterozygous mutations in Npt2a have been described in humans with nephrolithiasis and urinary phosphorus leak.[16]

The calcium-sensing receptor can influence phosphorus metabolism through connections with key hormones that regulate both calcium and phosphorus.[17] For example, a low serum calcium level detected by the calcium-sensing receptor stimulates release of parathyroid hormone, which increases urinary phosphorus excretion via downregulation of Npt2a.[18] A low serum calcium level also stimulates the production of calcitriol, which enhances absorption of both calcium and phosphorus through the gastrointestinal tract via actions on their selective ion channels.[19,20]

The SNP rs2970818 is located within 50 kb of *FGF6* and within 133 kb of *FGF23*. FGF6 is a paracrine factor that plays a

**Table 2.** Genetic loci associated with the serum phosphorus concentration in the discovery sample

| Locus | Top SNP ID | Position | Major/Minor Allele (MAF) | Meta | | In Gene | Closest Genes[b] |
|---|---|---|---|---|---|---|---|
| | | | | $\beta$[a] | *P* | | |
| 1p36.13 | rs1697421 | 21695879 | G/A (0.49) | +0.044 | $3.47 \times 10^{-16}$ | — | *ALPL*<br>*NBPF3* |
| 3q21.1 | rs17265703 | 123531334 | A/G (0.15) | −0.040 | $6.24 \times 10^{-08}$ | *CSTA* | *CASR*<br>*CCDC58* |
| 5q35.3 | rs4074995 | 176729949 | G/A (0.28) | −0.032 | $6.25 \times 10^{-08}$ | *RGS14* | *SLC34A1*<br>*PFN3*<br>*LMAN2*<br>*F12* |
| 6p21.31 | rs9469578 | 33814457 | C/T (0.08) | −0.064 | $5.15 \times 10^{-10}$ | *IHPK3* | *ITPR3*<br>*LEMD2*<br>*MLN* |
| 6q23.1 | rs453639 | 132091350 | A/C (0.36) | +0.036 | $3.59 \times 10^{-07}$ | *ENPP3* | |
| 6q23.2 | rs947583 | 136175352 | T/C (0.29) | +0.035 | $2.19 \times 10^{-09}$ | — | *PDE7B* |
| 12p13.32 | rs2970818 | 4476429 | T/A (0.09) | +0.052 | $4.04 \times 10^{-08}$ | — | *FGF6*<br>*RAD51AP1*<br>*FGF23*[c] |

MAF, minor allele frequency; *ALPL*, alkaline phosphatase, liver/bone/kidney; *NBPF3*, neuroblastoma breakpoint family, member 3; *CSTA*, cystatin A; *CASR*, calcium-sensing receptor; *CCDC58*, coiled-coil domain-58; *RGS14*, regulator of G-protein signaling 14; *SLC34A1*, solute carrier family 34 (sodium phosphate); *PFN3*, profilin 3; *LMAN2*, lectin, mannose-binding 2; *F12*, coagulation factor XII (Hageman factor); *IHPK3*, inositol hexakisphosphate kinase 3; *ITPR3*, inositol 1,4,5-triphosphate receptor, type 3; *LEMD2*, LEM domain containing 2; *MLN*, motilin; *ENPP3*, ectonucleotide pyrophosphatase/phosphodiesterase 3; *PDE7B*, phosphodiesterase 7B; *FGF6*, fibroblast growth factor 6; *RAD51AP1*, RAD51 associated protein 1; *FGF23*, fibroblast growth factor 23.
[a]Association with serum phosphate concentration in mg/dl.
[b]Within 50 kb.
[c]Distance = 133 kb.

role in myoblast proliferation and muscle differentiation;[21] associations of FGF6 with phosphorus metabolism have not been reported. Another fibroblast growth factor, FGF23, is an endocrine factor that is considered to be central to the regulation of phosphorus balance.[22] Originally identified as the cause of tumor-induced phosphorus wasting,[23] FGF23 binds to its receptor within the kidney to potently increase the urinary fractional excretion of phosphorus.[24] The hormone klotho is a key co-factor for FGF23; deletion of FGF23 or klotho in animal models yields a similar phenotype characterized by hyperphosphatemia, short-life span, vascular calcification, and osteoporosis.[25,26] Mutations in FGF23 are the cause of autosomal dominant hypophosphatemic rickets,[9] and elevated FGF23 levels are recently identified as the cause of phosphorus wasting in kidney transplant recipients.[27]

The most robust association in this study was for SNP RS1697421, which is located adjacent to the tissue-nonspecific alkaline phosphatase gene. Alkaline phosphatase is a membrane-bound enzyme that plays a key role in mineralizing the skeleton. In osteoblasts, alkaline phosphatase hydrolyzes pyrophosphate into two phosphate molecules, which are used to synthesize hydroxyapatite. Although this process will raise phosphorus levels within cells, the effect on the serum phosphorus concentration is unknown. Mutations in the alkaline phosphatase gene are linked with hypophosphatasia, a rare inherited condition characterized by failure to mineralize the teeth and bone, and excess circulating levels of pyrophosphate, a natural calcification inhibitor.[28] However, to our knowledge differences in the serum phosphorus concentration among individuals with hypophosphatasia have not been reported. Mutations in the *ENPP1* gene are linked with generalized arterial calcification of infancy, and polymorphisms in this gene are associated with insulin resistance and obesity.[29,30] Inositol hexakisphosphate kinase-3 is suspected to convert inositol hexakisphosphate to diphosphoinositol pentakisphosphate.[31]

Associations of individual genetic loci with the serum phosphorus concentration were modest. In aggregate, the seven top genetic loci identified in this study contributed to an approximate 0.4 mg/dl difference in the serum phosphorus concentration among the discovery sample. This variation is similar to that naturally observed between men and women and may be relevant for cardiovascular health. For example, each 0.5 mg/dl higher serum phosphorus concentration was linearly associated with an approximate 15% greater risk of cardiovascular events in the Framingham Heart Study and the Cholesterol And Recurrent Events study.[5,6]

Known intraindividual variation in serum phosphorus concentrations may have precluded identification of additional loci. Serum phosphorus concentrations vary throughout the day and from day to day, although they do not appear to be associated with fasting status.[32] In one study of 1878 participants in the National Health and Nutrition Examination Survey (NHANES) who underwent serum phosphorus measurements approximately 2 weeks apart, the correlation between serum phosphorus concentrations was 0.63.[13] In future studies, multiple measurements of serum phosphorus within an

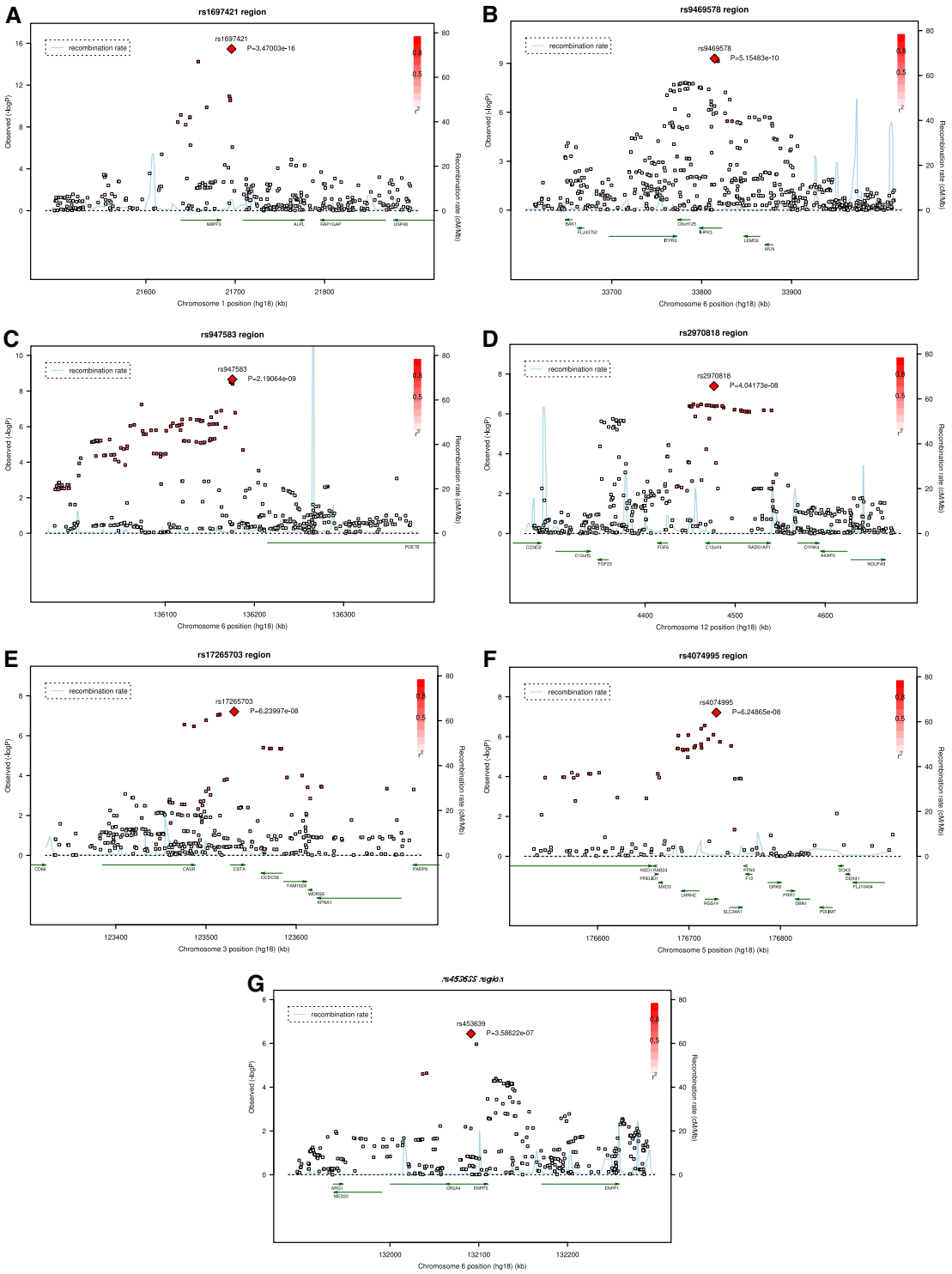


**Figure 2.** Seven loci meet genome wide significance level for the association with the serum phosphorus concentration. Observed log (*P* values) by chromosome position for genetic regions surrounding each top SNP in the discovery sample. Recombination rates are shown in red on the right-sided *Y*-axis. Correlation of the top SNP with surrounding SNPs indicated by shading—darker boxes indicate greater correlation (scale in top right-hand corner). (A) Locus 1p36.13; (B) locus 3q21.1; (C) locus 5q35.3; (D) locus 6p21.31; (E) locus 6q21.3; (F) locus 6q23.2; (G) locus 12p13.32.

individual would help reduce this source of variation. Our study was limited to Caucasian subjects; therefore, results do not apply to other race/ethnicities. The SNPs identified in this study do not imply a direct causal relationship with the serum phosphorus concentration; fine mapping and re-sequencing studies are needed to pinpoint potential causal variants, and

**Table 3.** Meta-analysis of the replication cohorts

| SNP ID | Replication *n* | Power[a] | Discovery $\beta$ | Replication $\beta$ | Replication *P* | Combined $\beta$[b] | Combined *P*[b] |
|---|---|---|---|---|---|---|---|
| rs1697421 | 5536 | 0.999 | +0.044 | +0.070 | $2.08 \times 10^{-14}$ | +0.050 | $1.14 \times 10^{-27}$ |
| rs17265703 | 5543 | 0.730 | −0.040 | −0.026 | 0.015 | −0.036 | $4.32 \times 10^{-9}$ |
| rs4074995 | 5497 | 0.734 | −0.032 | −0.010 | 0.168 | — | — |
| rs9469578 | 5544 | 0.978 | −0.064 | −0.046 | 0.004 | −0.059 | $1.11 \times 10^{-11}$ |
| rs453639 | 5544 | 0.409 | +0.036 | +0.014 | 0.096 | — | — |
| rs947583 | 5543 | 0.921 | +0.035 | +0.034 | $3.94 \times 10^{-4}$ | +0.035 | $3.45 \times 10^{-12}$ |
| rs2970818 | 5544 | 0.760 | +0.052 | +0.034 | 0.025 | +0.047 | $4.38 \times 10^{-9}$ |

[a]Power to replicate findings from the discovery cohorts.
[b]Combined results shown for replication $P < 0.05$.

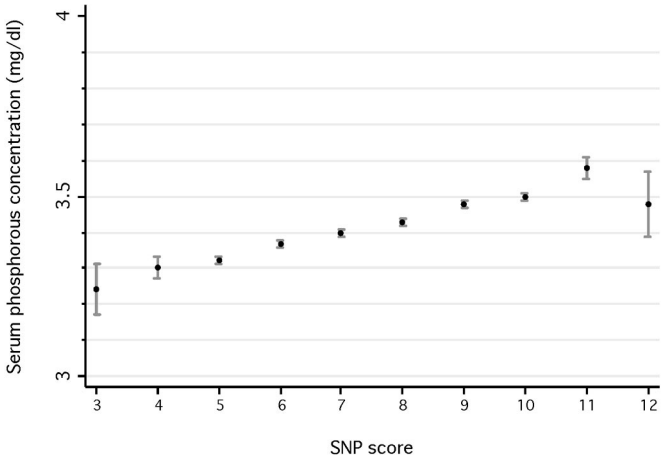


**Figure 3.** Mean serum phosphorus concentrations associate with the number of SNPs individually associated with serum phosphorous. Mean serum phosphorus concentration as a function of the number of risk alleles in the discovery sample; number of participants with the risk alleles shown below the graph.

functional studies are needed to understand how the specific genetic variation may be realized at the protein level. Because of known selection bias in selecting SNPs on the basis of genome-wide statistical significance and relatively low allele frequencies, study power to replicate associations for loci 3q21.1, 5q35.3, 6q23.1, and 12p13.32 was inadequate. $\beta$ coefficients for SNPs within these loci were smaller in magnitude, but in the same direction as those from the discovery cohort. Finally, this genome-wide association study is limited to detecting common genetic variants and is therefore unable to identify associations for rare alleles, such as those responsible for phosphorus wasting disorders in humans.

In summary, we demonstrate that common genetic variants are associated with the serum phosphorus concentration using meta-analysis of study-specific GWAS from four large adult populations. Follow-up studies are needed to identify potential causal genetic loci in or near the candidate genes identified in this GWAS. Candidate genes may be explored in more comprehensive metabolic studies of phosphorus metabolism and in translational animal models that could shed new light on the mechanisms and clinical implications of phosphorus homeostasis.

## CONCISE METHODS

### CHARGE Consortium

The Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium was developed to facilitate the conduct and replication of genetic association studies across established cohort studies.[33] Each participating study approved guidelines for collaboration, and the institutional review boards for each study approved the consent procedures, data security measures, and collection of genetic material. All study participants provided written informed consent for genetic research.

### Discovery Study Populations

Serum phosphorus measurements were available from four participating discovery cohorts within the CHARGE consortium: the Cardiovascular Health Study (CHS), the Framingham Heart Study (FHS), the Atherosclerosis Risk in Communities Study (ARIC), and the Rotterdam Study (RS).

CHS is a community-based study of cardiovascular disease among adults ages 65 years and older.[34] CHS recruited 5201 ambulatory older adults in 1989 to 1990 from four U.S. communities: Forsyth County, NC; Sacramento County, CA; Washington County, MD; Pittsburgh, PA. Subjects were excluded if they were institutionalized, required a wheelchair in the home, or were undergoing treatment for cancer. Serum phosphorus concentrations and genomic data were available for 2337 CHS participants without cardiovascular disease at the time of 1992 to 1993 exam.

FHS is a prospective cohort study initiated in 1948 to investigate risk factors for cardiovascular disease. Children and spouses of children from the original cohort were recruited in 1972 (Framingham Offspring Study) and followed prospectively with clinic examinations every 4 years.[35] Serum phosphorus concentrations and genomic data were available for 2865 participants during the second examination cycle in 1979 to 1982.[5]

ARIC is a community-based, prospective cohort study of atherosclerosis that recruited 15,792 Caucasian and African-American individuals aged 45 to 64 years from four U.S. communities: Forsyth County, NC, Washington County, MD, Minneapolis, MN, and Jackson, MS.[36] Participants underwent three follow-up examinations at 3-year intervals. Serum phosphorus concentrations and genomic data were available for 8122 ARIC participants from the baseline examination in 1987 to 1989.

RS is a community-based cohort study to assess determinants of chronic diseases among the elderly that recruited 7983 participants (78% of the eligible population) aged ≥55 years living in a well-defined suburb of the city of Rotterdam, The Netherlands, in 1990 to 1993.[37] Home visits were used to collect health status information. Participants were subsequently examined at the research center in 1990 to 1993 and every 3 to 4 years thereafter. Serum phosphorus concentrations and genomic data were available for 3516 RS participants.

Because of the relatively small number of non-Caucasian participants, we limited analyses to participants of primarily European (Caucasian) ancestry. Previous studies have demonstrated that serum phosphorus concentrations increase markedly in advanced stages of chronic kidney disease.[38] We therefore excluded participants who had an estimated GFR <45 ml/min per 1.73 $m^2$ to focus on potential factors that regulate phosphorus under normal conditions. We estimated GFR using the four-variable Modification of Diet in Renal Disease equation.[39]

## Replication Study Populations

KORA S3 is an independent population-based sample from the general population living in the region of Augsburg, Southern Germany, in 1994 to 1995. A total of 3006 subjects participated in a follow-up examination in 2004 to 2005 (KORA F3). The KORA S4 survey (4261 participants) is an independent population-based sample from the same region and was conducted in 1999 to 2001.[40] Serum phosphorus concentrations and genomic data were available for 1599 participants of KORA F3 and for 1779 participants of KORA F4. The Vis study was conducted in 2003 to 2004 among 986 unselected, 18- to 93-year-old Croatians recruited from the villages of Vis and Komiza, on the Dalmatian island of Vis. These settlements have unique population histories and have preserved isolation from other villages and from the outside world for centuries. Health ABC is a prospective cohort study designed to investigate the effect of health conditions on age-related functional changes. Participants aged 70 to 79 years were recruited from the metropolitan areas surrounding Pittsburgh, PA, and Memphis, TN. Eligibility criteria were no difficulty walking one-quarter of a mile, climbing 10 steps, or performing basic activities of daily living.

## Serum Phosphorus Measurements

Blood specimens were collected in the morning, centrifuged, and either frozen at −70°C for storage or run daily; not all participants were fasting. Serum phosphorus concentrations were quantified using an automated platform (Beckman-Coulter for CHS and ARIC, Roche for FHS, and a Kone Diagnostica reagent kit and a Kone autoanalyzer for RS) in which inorganic phosphorus reacts with ammonium molybdate in an acidic solution to form a colored phosphomolybdate complex. The reported intra-assay coefficients of variation for these assays are 5.8 and 5.6%, respectively. Intraindividual biologic variation in serum phosphorus measurements are available from a previous population-based cohort study in which 1878 participants underwent repeat serum phosphorus measurements a median of 16 days apart.[13] The correlation between measurements was 0.63, and the SD for the difference in measurements was 0.31 mg/dl.

## Genotyping

*CHS.*

Genotyping was performed at the General Clinical Research Center's Phenotyping/Genotyping Laboratory at Cedars-Sinai using the Illumina 370CNV BeadChip system. Genotypes were called using the Illumina BeadStudio software. Samples were excluded for genotypic sex mismatch, discordance with prior genotyping, or call rate <95%. Genotyping was successful in 96%. The following exclusions were then applied: call rate <97%, Hardy Weinberg Equilibrium $P < 10^{-5}$, duplicate error or Mendelian inconsistency, heterozygote frequency of approximately 0, or SNP not found in dbSNP. SNPs were further excluded from analysis if the ratio of the variance of the allele dosage to the variance expected under Hardy Weinberg Equilibrium was <0.01.

*ARIC.*

Genotyping was performed using the Affymetrix 6.0 array and genotypes were called using the Birdsees software. Of 8861 samples, 734 were excluded because of discordance with previous genotype data, mismatch of reported and genotypic sex, first-degree relative of an included individual based on genotype data, or genetic outliers as assessed by average allele sharing and principal components analyses. Before imputation, SNPs with call rate <95%, minor allele frequency <1%, and Hardy-Weinberg equilibrium $P < 10^{-5}$ were excluded to ensure good quality for imputation. After imputation, no SNPs were excluded.

*FHS.*

Genotyping was conducted using the Affymetrix 500K mapping array and the Affymetrix 50K gene-focused macrophage inflammatory protein array. Individuals were excluded when their call rate across all SNPs was <97%. After exclusion of SNPs with genotype call rate <95% or Hardy-Weinberg equilibrium $P < 10^{-6}$, there were 503,551 SNPs available for analysis.

*RS.*

Genotyping was done with the Illumina 550K array (Illumina, San Diego, CA, USA) in self-reported Caucasian individuals. Individuals were excluded for an overall call rate <97.5%, excess autosomal heterozygosity, mismatch between genotypic and phenotypic sex, or outliers identified by the identity-by-state clustering analysis; genotyping was successful in 93% of RS. SNPs were excluded when the minor allele frequency was ≤1%, Hardy-Weinberg equilibrium $P < 10^{-6}$, or SNP call rate ≤98%, resulting in 530,683 directly measured SNPs used for imputation.

## Imputation

In all studies, genotypes were imputed to approximately 2.5 million SNPs in HapMap, using the Phase II CEU individuals as a reference panel. For imputation software, ARIC, FHS, and RS used MACH and CHS used BIMBAM.[41,42] Imputation results are summarized as an "allele dosage" (a fractional value between 0 and 2), defined as the expected number of copies of the minor allele at that SNP.

## Statistical Analysis

We evaluated serum phosphorus concentrations as a continuous variable, in mg/dl. The distribution of serum phosphorus concentrations

was not strongly skewed and was analyzed without transformation. We did not exclude any individuals based on their serum phosphorus concentration.

Genome-wide analyses were conducted within each cohort. Using an additive genetic model, we used linear regression to evaluate the association between the allele dosage and serum phosphorus concentration, quantifying the regression slope ($\beta$) and standard error [SE($\beta$)]. We adjusted analyses for age, sex, and study site. We accounted for relatedness of individuals in FHS using random effects to account for the covariance between family members with the specific covariance structure determined by the degree of relatedness between each relative pair.[33] Within each study, genomic control was used to adjust each study's standard errors for potential effects of population stratification; the genomic control $\lambda$ values ranged from 1.01 to 1.04 across the discovery and replication cohorts. In all cohorts except for Health ABC, principal components were not associated with the serum phosphorous concentration. In Health ABC, principal components were associated with serum phosphorus; therefore, we adjusted for principal components in Health ABC to account for population substructure. We combined within-study associations by meta-analysis, using inverse variance weighting. After meta-analysis, we filtered results on weighted minor allele frequency $<0.005$. We selected an *a priori* genome-wide significance threshold of $4 \times 10^{-7}$, which corresponds to a $\leq 1$ expected false-positive result for 2.5 million tests. The validity of this boundary is not affected by correlation between test statistics.[43]

To assess interactions, we used a genotype-sex interaction parameter within the same linear modeling and meta-analysis framework. To summarize the main effects from the discovery sample, we constructed an additive gene score model using the number of copies of each risk allele from each SNP (possible values 0 to 14) and used linear regression to evaluate the association of gene score with serum phosphorus concentration.

We estimated power to replicate observed associations from the discovery phase in a replication sample size of 5544, an observed SD for phosphorus of 0.5, and a one-sided $\alpha$ of 0.05. Allele frequencies were based on the discovery cohorts and effect sizes were adjusted for the "winners curse" selection bias.[44] Because of this adjustment and relatively low minor allele frequencies, power was limited to replicate findings in loci 3q21.1, 5q35.3, 6q23.1, and 12p13.32.

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## DISCLOSURES

None.

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