Heritability and Multipoint Linkage Analysis Suggest the Contribution of Angiopoietin-TEK Pathway to the Variation of Serum Creatinine and Glomerular Filtration Rate

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Abstract

Background: Serum creatinine and estimated glomerular filtration rate (eGFR) are keys to assess kidney function and suggest a propensity for development of renal failure. Genetic factors are reported in different ethnic groups to play a role in the variability of these phenotypes. In this study, we investigate the heritability and quantitative trait loci (QTL) of serum creatinine and eGFR. Multiple phenotypes were collected from a multi-generation pedigree of 281 subjects form Oman family study. Genotype analysis was used to calculate heritability and linkage was performed using variance components decomposition-based methods implemented in SOLAR.

Results: The multivariate-adjusted heritability estimates for serum creatinine and eGFR were 0.70 and 0.63 (p-value 2.5x10-11 and 1.8x10-11), respectively. Genome-wide linkage analysis showed significant loci with LOD score ≥2 at chromosomes 9p21.1, 9p21.3, 15p26.3 and 16.p13.3. Functional annotation identified angiopoietin 1-TEK pathway as a candidate pathway where TEK is associated with chronic kidney disease and expressed in renal glomeruli.

Conclusion: This study showed high heritability of serum creatinine and eGFR with significant QTLs in chromosomes 9, 15 and 16. Further research is needed to study the genetic association studies of angiopoietin 1-TEK axis with glomerulopathies like type 2 diabetes and to predict and diagnose progression of renal diseases.

Introduction

End-stage renal diseases (ESRDs) are strongcontributors to morbidity and mortality worldwide(Mortality & Causes of Death, 2016).Patients with ESRD are more susceptible to associated complications including being at higher risk of cardiovascular disease development (Baigent, Burbury, & Wheeler, 2000). Serum creatinine and estimated glomerular filtration rate (eGFR) arefrequently used to measure kidney functionto serve in diagnosis, to monitor disease progression, and evaluate therapeutic responses. Serum creatinine, produced by creatine phosphate metabolism, reflectseGFR to a reasonable extent and confounded by factors such as muscle mass, age, race, and gender (Stevens et al., 2008).

The intricacy of genetic and environmental factors has been previously investigated in relation to serum creatinine and eGFR(Fox et al., 2004; Langefeld et al., 2004; Thameem et al., 2013). Genome-wide linkage analyses (GWAS) of kidney function conducted in a range of populations identified genomic loci variants associated with kidney traits, including serum creatinine and eGFR variations(Fox et al., 2004; Hwang, Yang, Meigs, Pearce, & Fox, 2007; Kottgen, 2010; Liu et al., 2011; Moulin et al., 2017).Evidence across various ethnic groups identifiedpositional candidate genes that influence measures of renal function, their functional significance remains to be investigated. Establishing the heritability of renal function parametersin different ethnic groups would reinforce the phenotypic variabilityand could suggest genetic factors in estimation of renal filtration capacity. This study aimed to measure the heritability (h^2R) and

identify quantitative trait locus (QTL) of serum creatinine and eGFR in an Arab ethnic population using family-based measured genotype analysis.

Results

Characteristics of participants and pedigree analyses

The structure of the pedigree and the relationship between members were shown in Table 1. The pedigree consisted of 23 sub-pedigrees with intermarriages and a total of 148 nuclear families with 109 founder individuals. The pedigree was highly extended with maximum relation reached to the 9-degree kinship. The mean age was 31 years, 53% (n=149) were females and 47% (n=132) were males.Gender differences were observed in serum creatinine (male 73.33±17.29 vs female 47.68±10.32) and eGFR (male 120.54 ±36.67 vs female 142.34±38.81, *p-value*<0.001, Table 2.

Parameters that correlate with serum creatinine and eGFR

In the polygenic model covariates are used to strengthen the estimation of heritability and quantitative trait loci. To identify covariate, we run correlation matrix of all phenotypes against serum creatinine and eGFR, Figure 1. Serum creatinine had strong correlation with eGFR (r-coefficient -0.77, p-value <0.0001), Figure 1. It had weaker correlation with age, serum triglyceride, BMI and waist circumference. eGFR had moderate correlation with age (r-correlation coefficient -0.48, p-value <0.0001) and weak correlation with total cholesterol, triglyceride, body mass index (BMI) and waist circumference. Fasting blood sugarwas not significantly correlation with serum creatinine and eGFR, Figure 1.From the correlation analysis, we selected total cholesterol, serum triglycerides, BMI,waist circumference and age along with gender as covariates for further measured genotype analysis.

Heritability of Serum Creatinine and eGFR

We analysed the pedigree of 281 subjects to estimate the crude and adjusted heritabilityofserum creatinine and eGFR using variance component analysis in SOALR. Following covariates were used to adjust the heritability: age, gender, total serum cholesterol, serum triglycerides, BMI, and waist circumference. Heritability (H^2R) of serum creatinine was 0.64±0.19 (p-value $1.0x10^{-7}$) andwith covariate adjustment theH^2R was increased 0.70±0.13 (p-value $2.5x10^{-11}$), Table 3. While the H^2R of eGFR was 0.37±0.12 (p-value $2.1x10^{-6}$) and with adjustment 0.63±0.13 (p-value $1.8x10^{-11}$), Table 3.

Multipoint Quantitative Linkage Analyses of serum creatinine and eGFR

We use measured genotype analysis in SOLAR to identify quantitative trait loci (QTL) that determine the heritability of serum creatinine and eGFR. We first analyzed the statistical power of the pedigree and the model to identify a significant QTL. Using the current genealogical information, Figure-2a shows that the pedigree had 80% power to detect a locus with a significant logarithm of the odds (LOD) score of ≥2 with a trait heritability of ~0.28 and locus of LOD score of 3 with heritability of 0.24. These values are lower

than the measured heritability of serum creatinine and eGFR (Table 3), therefore the pedigree model has higher power to detect significant loci of LOD score of 2 and above.

To identify QTLs, each individual was genotyped at 343 microsatellite markers for a 10 cM genome-wide scan.Since eGFR calculation involve serum creatinine, we observed overlapping multipoint genome-wide linkage peaks, Figure 2b-d. We found significant QTLs with LOD scores of ≥2 with highest LOD score for serum creatinine was 2.43 on chromosome 16p13.3 at 25 cMbetween markersD16S2613 and D16S3047 (Table 4, Figure 2d) while for eGFR , a LOD score of 2.71 was detected at chromosome 15p26.3 at 138 cM between the markers D15S87 and D15S642, Table 4 and Figure 2c. Two peaks were found at chromosome 9p21.1-p21.3 (44 and 51 cM) between D9S171 and D9S1853 markers, Table 4 and Figure 2b.

Gene Mapping of significant quantitative trait loci:

We performed bioinformatics search for genes located in the microsatellite region that showed significant LOD scores listed in Table 4. Total of 25 genes were identified in the chromosomal regions of significant linkage with serum creatinine and eGFR. Table 5 describes any reported genetic association with chronic kidney disease (GAD-CKD) and renal expression from two expression databases Unigene-EST and CGAP-SAGE. TEK receptor tyrosine kinase (TEK) gene at chromosome 9 was found to have genetic association with chronic kidney disease and reported expression in normal and cancerous renal tissues.

Discussion

In this study, we examined the genetic contribution on serum creatinine and eGFR, known parameter of kidney function, and identified the quantitativeloci that contribute to the genetic variations of these parameters. With a family-based model adjusted for age, gender, total cholesterol, serum triglycerides, BMI and waist circumference as covariates, we found 63% and 70% of variations in eGFR and serum creatinine, respectively, are due to genetic component. We further identified four significant loci contributing to the heritability of kidney function parameters: 9p21.1, 9p21.3, 15p26.3 and 16.p13.3.

Our study reported high heritability values for serum creatinine and eGFR using a large multi-generation pedigree adjusted to multiple confounding variables. In type 2 diabetic patients, heritability of GFR was found high as 75% with adjustment of age, sex, mean arterial blood pressure, medications, and glycated hemoglobin(Langefeld et al., 2004). In the Framingham Heart Study, genome wide linkage analysis of 330 nuclear families found heritability of serum creatinine as 29% and 33% for eGFR adjusted for age, gender, BMI, diabetes, systolic BP, hypertension treatment, tobacco use, and HDL cholesterol(Fox et al., 2004). In the Swedish Twins-based study, the heritability of serum creatinine was reported as 19%, creatinine based-eGFR as 18.6% and Cystatin-C-based eGFR as 41.8% (Arpegard et al., 2015). Another United Kingdom twin study reported heritability of creatinine of 37% and eGFR as 63% (Hunter et al., 2002). The differences in reported heritability of serum creatinine and eGFR is attributed to the ethnicity of participants under the study or inclusion of patient population. Altogether, these studies suggest strong genetic component in the variability of serum creatinine and eGFR. This provide insights to find the genes

that are associated with these biochemical measures and improve the understanding in the variation of kidney function within a population.

we used a validated genetic model of extended pedigree structure with heritability estimation extended up to 9th degree kinship. We identify QTLs for serum creatinine and eGFR in three chromosomes 9, 15 and 16. Prior genome-wide linkage studies identified various QTLs influencing serum creatinine-based eGFR, but none reported significant LOD scores on chromosomes 9, 15, and 16 (Hunt et al., 2004). In Strong Heart Family study, linkage analysis identified significant association between eGFR and SNPs within *SLC6A13* (ch.12p13.33),*UBE2Q2* (ch.15q24.2), *PIP5K1B* (ch19p12), and *WDR72* (ch15q21.3)genes(Franceschini et al., 2014). One of the candidate gene in contributing to genetic variability in kidney function is Tyrosine kinase receptor (*TEK*) that ismainly expressed on endothelial cells and serves as a receptor for angiopoietin-1 (*ANGPT1*) (Suri et al., 1996). Glomerular capillaries are composed of a fenestrated endothelium and specialised epithelial cells (the podocytes).ANGPT1-induced TEK signalling pathway regulates cell survival, quiescence, proliferation, migration, adhesion, and vascular permeability (Suri et al., 1996).Therefore, the role of angiopoietins is important in glomerular function in healthy and disease state(Gnudi, 2016). ANGPT1is constitutively expressed in podocytes(Satchell et al., 2002) while TEK expression is localised in the endothelium of mouse glomerular capillaries (Yuan, Suri, Yancopoulos, & Woolf, 1999) and podocyte of rat kidneys(Dessapt-Baradez et al., 2014).. Moreover, heterozygous deletion of TEK in mice results in reducedglomerular vasculature(Puri, Partanen, Rossant, & Bernstein, 1999). In human, defects in TEK causes an autosomal dominant form of venous malformation (Fukuhara et al., 2008; Wouters et al., 2010) which manifests with abnormalities in kidney function (Cura, Elmerhi, Suri, Bugnone, & Dalsaso, 2010).

Conclusion

Our study reported high genetic component of serum creatinine and eGFR with significant contribution of loci in chromosomes 9, 15 and 16 to the heritability of these parameters. We suggest future genetic association studies of ANGPT1-TEK axis with diseases associated with glomerulopathies like type 2 diabetes (Dessapt-Baradez et al., 2014).

Methods

Study Population: Oman Family Study

Data of the study participants was all obtained from Oman Family study, a family-based genetic model (Bayoumi et al., 2007; Zadjali, Al-Yahyaee, Hassan, Albarwani, & Bayoumi, 2013). The data included family medical history, demographic data, and lifestyle. Out of five highly consanguineous families, one family consisting of 281 individuals was selected because of availability of serum creatinine and eGFR measurements. All methods were carried out in accordance with relevant guidelines and regulations. The study was approved by the ethics committee in Sultan Qaboos University and written informed consent was obtained from each participant.

Measurement of phenotypic data

Multiple phenotypic data were collected in Oman family study, described earlier (Hassan et al., 2011; Zadjali et al., 2013). Body weight and height were used to calculate body mass index (BMI), and waist circumference was measured. Blood samples were obtained from overnight fasted subjects in non-additive tubes (Becton Dickinson and Company, USA) and serum was obtained after centrifugation. Serum creatinine were measured along other biochemical parameters using Rocha Cobas Integra-400 (Roche Diagnostics, Germany). Total cholesterol, total serum triglycerides, blood glucose, and lipoproteins levels were assayed by enzymatic calorimetric methods. Serum creatinine was corrected using the Jaffe method using following formula corrected-creatinine = 1.058 × (serum creatinine) – 15.951. GFR was estimated using the Modification of Diet in Renal Disease equation (MDRD)(Levey et al., 1999): eGFR (mL/min/1.73 m2) = 175 × (Corrected serum creatinine µmol/L x 0.0113)$^{-1.154}$ × (Age)$^{-0.203}$× (0.742 if female)

Descriptive statistics:

Baseline characteristics were analyzed using IBM SPSS statistics v.21. and expressed as mean ± standard deviation. To test the normal distribution, Kolmogorov-Smirnov test was carried out. Mann-Whitney U test was performed for non-normal distributed data. A two tailed P <0.05 was considered significant. Pearson correlation and scatter plot matrix were generated using R statistical software (Foundation for Statistical Computing, Vienna, Austria).

Heritability and genome-wide quantitative linkage analysis:

Heritability is defined as the ratio of genetic variability in traits attributable to individual alleles(Zuk, Hechter, Sunyaev, & Lander, 2012). To estimate crude h^2R for serum creatinine and eGFR, we used the Sequential Oligogenic Linkage Analysis Routines (SOLAR) software package (Almasy & Blangero, 1998; Amos, 1994). The expected effect of all measured covariates on eGFR was further computed. To achieve normality, data that showed kurtosis by redefining the traits using normalization function using SOLAR.

To perform multipoint linkage analysis, we first determined the pedigree power to determine a significant locus of LOD score of 2 and 3 at different trait heritability values. Power calculation was performed using the Sequential Oligogenic Linkage Analysis Routines (SOLAR) software package (Version 4.1.7)(Almasy & Blangero, 1998). Subsequently, we collected the genotype data for each subject analyzed previously (Hassan et al., 2011). Briefly, genotype results were collected for 343 microsatellite markers for a 10 cM genome-wide scan by the Mammalian Genotyping Services at Marshfield Clinic Research Foundation (http://research.marshfield- clinic.org/genetics). Identity by descent was calculated using all information provided in the pedigree to scan for allele sharing probabilities between the individuals. Genotype information was used to determine quantitative trait loci (QTL); loci associated with phenotypic variance and characterized by related individuals sharing 0, 1, or 2 alleles (Fox et al., 2004). Logarithm of odds (LOD) scores were calculated and adjusted with the covariates for each trait in order to perform

multipoint quantitative linkage analysis (SOLAR). LOD scores ³2 were considered significant (Almasy & Blangero, 1998).

Functional annotations of Genes under QTL regions:

We collected the list of genes within the significant QTL regions that determines serum creatinine and eGFR heritability. We searched for gene-disease mappings Genetic Association Database for any kidney disease (GAD-CKD) (Becker, Barnes, Bright, & Wang, 2004) and also search for tissue expression using two databases: Unigene-EST and Cancer Genome Anatomy Project (CGAP)-serial analysis of gene expression (CGAP-SAGE) (Boon et al., 2002).

Abbreviations

ANGPT1: angiopoietin-1; BMI: Body Mass Index; cM: centimorgan;eGFR: estimated glomerular filtration rate; ESRDs: End-stage renal diseases; GAD-CKD : genetic association with chronic kidney disease; H^2R : heritability; LOD: logarithm of the odds; QTL: quantitative trait loci; TEK: TEK receptor tyrosine kinase

Declarations

Ethics approval and consent to participate:

All methods were carried out in accordance with relevant guidelines and regulations. The study was approved by the ethics committee in Sultan Qaboos University and written informed consent was obtained from each participant.

Consent for publication

Not Applicable.

Availability of data and materials

The raw datasets (pedigree files and microsatellite genotypes) used and analyzed during the current study are not depositedin publicly available repositories because of considerations about the security ofhuman genetic resources but the data is available upon request to the corresponding author.No sequence or array files are generated form the study to be deposited in relevant databases.

Competing interests

The authors have declared no competing interests.

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Authors' contributions

FZ, M.O.H, R.A.B, S.Alb, S.A designed the study. F.Z designed the method of analysis.F.Z and M.A performed the analyses. FZ, M.O.H, R.A.B, S.Alb, S.A, N.B.A and RZ assisted with interpretation. F.Z wrote the manuscript. All authors reviewed the manuscript. The authors read and approved the final manuscript.

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Consent for Participation

Not applicable

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Tables

Table 1: descriptive analysis of pedigree structure

	Number
Sub-pedigree	23
Nuclear families	148
Founders	109
Number of pedigree relation pairs	651
Parent- offspring	
Siblings	438
grandparent- grandchild	980
Avuncular	1030
Half sibling	159
3rd degree	5252
4th degree	7210
5th degree	6794
6th degree	2592
7th degree	755
8th degree	301
9th degree	59

Table 2: Descriptive characteristics of pedigree members

	Total	Male	Female	p-value
Number	281	149 (53.0 %)	132 (47.0 %)	
Age (years)	31(15)	28 (13)	33 (17)	n.s
Fasting blood glucose (mmol/L)	5.48 (1.37)	5.31 (0.59)	5.64 (1.78)	n.s
Total Cholesterol (mmol/L)	4.54 (1.04)	4.36 (0.94)	4.71 (1.10)	< 0.01
Serum Triglycerides (mmol/L)	1.17 (0.82)	1.31 (0.80)	1.05 (0.83)	< 0.001
Serum creatinine (µmol/L)	59.68 (18.98)	73.33 (17.29)	47.68 (10.32)	< 0.001
eGFR (mL/min/1.73 m^2)	132.14 (39.30)	120.54 (36.67)	142.34 (38.81)	< 0.001*
Body Mass Index (Kg/m^2)	24.70 (6.43)	24.99 (7.42)	24.43 (5.39)	n.s
Waist Circumference (cm)	78.41 (15.09)	80.28 (14.90)	76.74 (15.11)	< 0.05*

Data are shown with mean (± standard deviations). Whitney U test was used as statistic test for skew distributed phenotypes. *normally distributed phenotypes analysed byStudent t test. n.s : statistically not significant.

Table 3. Heritability of serum creatinine and eGFR

	Serum Creatinine		eGFR	
	H^2R (SE)	p-value	H^2R (SE)	p-value
Crude	0.64 (0.19)	$1.0x10^{-7}$	0.37 (0.12)	$2.1x10^{-6}$
adjusted	0.70 (0.13)	$2.5x10^{-11}$	0.63 (0.12)	$1.8 x10^{-11}$

Heritability was adjusted using following covariates: age, gender, total serum cholesterol, serum triglycerides, BMI, and waist circumference

Table 4: Genome-wide scan for serum creatinine and eGFR

Chr.	peak cM	markers	serum Creatinine	eGFR
			LOD	LOD
9p21.1	44	D9S171-D9S1853	2.33	2
9p21.3	51	D9S171-D9S1853	2.28	-
15p26.3	138	D15S87-D15S642	2.29	2.71
16p13.3	25	D16S2613 - D16S3047	2.43	2.25

LOD: log of the odds ratio. LOD score of ≥2 considered significant. cM: centimorgan position in each chromosome.

Table 5: Genes identified in linked regions of chromosomes 9, 15 and 16.

Gene	Name	Chr.	GAD-CKD	Unigene EST	CGAP-SAGE
MOB3B	MOB kinase activator 3B	9		+	+
IFT74-AS1	IFT74 antisense RNA 1	9			
EQTN	equatorin	9			
IFNK	interferon kappa	9		+	
IFT74	intraflagellar transport 74	9			
PLAA	phospholipase A2 activating protein	9			+
TEK	TEK receptor tyrosine kinase	9	+	+	+
IZUMO3	IZUMO family member 3	9			
CAAP1	caspase activity and apoptosis inhibitor 1	9			
MIR876	microRNA 876	9			
TUSC1	tumor suppressor candidate 1	9			
LINGO2	leucine rich repeat and Ig domain containing 2	9			
LRRC19	leucine rich repeat containing 19	9		+	
MIR873	microRNA 873	9			
SNRPA1	small nuclear ribonucleoprotein polypeptide A	15			+
ASB7	ankyrin repeat and SOCS box containing 7	15		+	
ALDH1A3	aldehyde dehydrogenase 1 family member A3	15			
LRRK1	leucine rich repeat kinase 1	15			
TARSL2	threonyl-tRNA synthetase like 2	15			
TM2D3	TM2 domain containing 3	15		+	+
CHSY1	chondroitin sulfate synthase 1	15			
LINS	lines homolog 1	15			
RSL1D1	ribosomal L1 domain containing 1	16			+
GSPT1	G1 to S phase transition 1	16			
PCSK6	proprotein convertase subtilisin/kexin	16			+

	type 6	
SNN	stannin	16
BCAR4	breast cancer anti-estrogen resistance 4	16
LITAF	lipopolysaccharide induced TNF factor	16

Figures

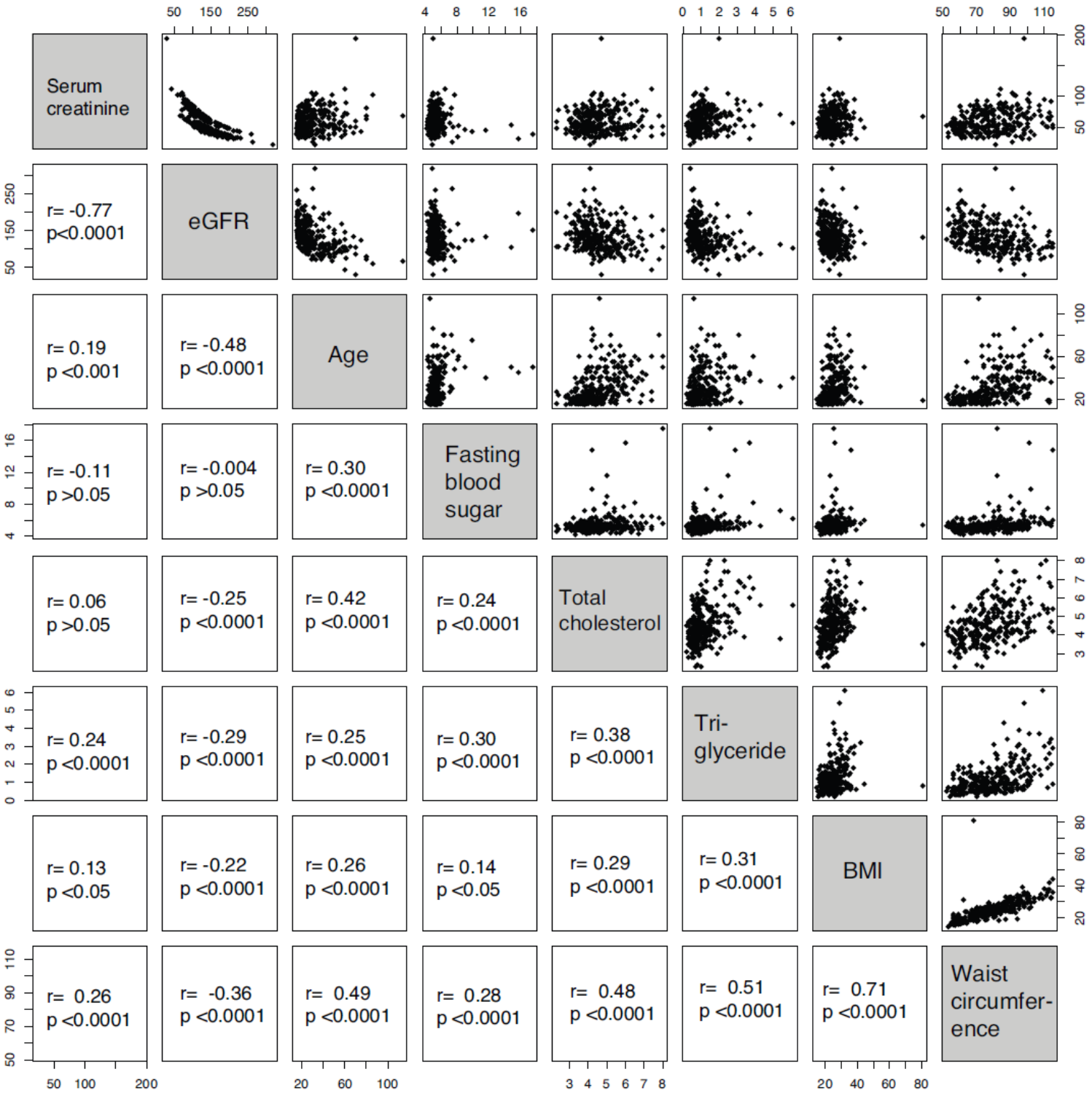

Figure 1

Scatter plot matrix of serum creatinine and estimated-glomerular Filtration rate (eGFR) and metabolic parameters.Pearson correlation coefficient (r) and p-value are shown in the box of the lower panel. BMI: Body Mass Index

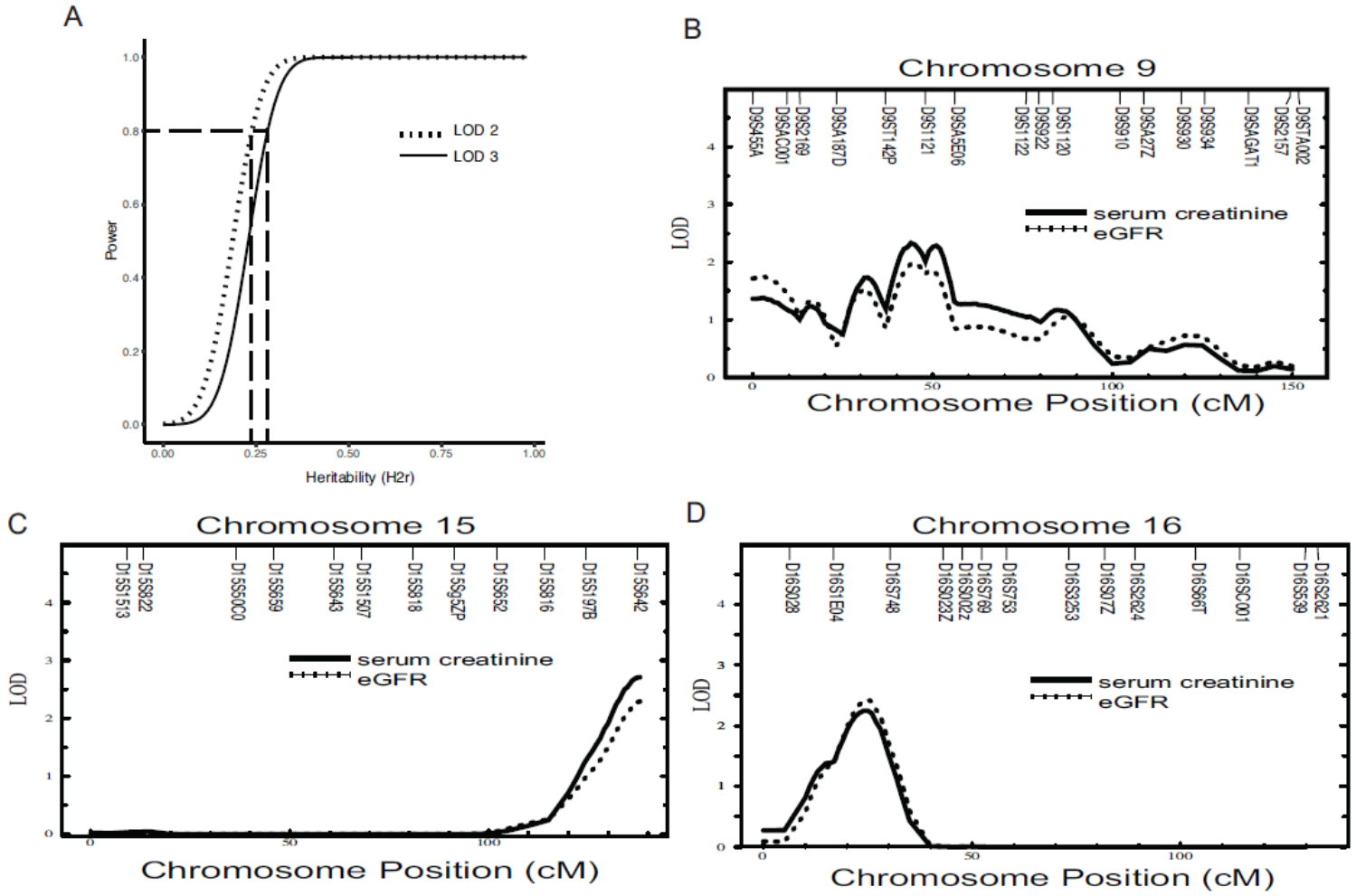

Figure 2

Multipoint quantitative linkage analysis of serum creatinine and eGFR. (A) Pedigree power analysisat significant LOD scores of 2 and 3 for traits of different heritability (0-1). Quantitative linkage analysis with significant LOD score of ≥ 2 for chromosomes 9 (B), 15 (C) and 16 (D).