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Abstract

Fibroblast growth factor 1 gene - FGF1 - is expressed primarily in the kidney and is postulated to contribute to hypertension. However, the biological mechanisms underlying the association between FGF1 and blood pressure regulation remain unknown.

We report that the major allele of FGF1 single nucleotide polymorphism rs152524 was associated in a dose-dependent manner not only with systolic blood pressure ($P=9.65x10^{-5}$) and diastolic blood pressure ($7.61x10^{-3}$) in a meta-analysis of 14364 individuals but also with renal expression of FGF1 mRNA in 126 human kidneys ($9.0x10^{-3}$). Next-generation RNA-sequencing revealed that renal up-regulation of FGF1 expression globally and of each of its 3 mRNA isoforms individually is associated with higher blood pressure. FGF1-stratified co-expression analysis in 2 separate collections of human kidneys identified 126 FGF1 partner mRNAs, of which 71 and 63 showed at least nominal association with systolic and diastolic blood pressure, respectively. Of those, 7 mRNAs in 5 genes (MME, PTPRO, REN, SLC12A3 and WNK1) had strong prior annotation to blood pressure or hypertension. MME (that encodes an enzyme responsible for degradation of circulating natriuretic peptides) showed the strongest differential co-expression with FGF1 between hypertensive and normotensive kidneys. Higher level of renal FGF1 expression was associated with lower circulating levels of atrial and brain natriuretic peptides.

These findings indicate that FGF1expression in the kidney is at least under partial genetic control and that renal expression of several FGF1 partner genes in natriuretic peptides catabolism pathway, renin-angiotensin cascade and sodium handling network may explain the association between FGF1 and blood pressure.

Introduction

Essential hypertension is a net product of genetic factors and environmental exposure acting together on regulatory systems in key organs for blood pressure (BP) homeostasis. The kidney is central to BP regulation and drives the development of hypertension through numerous mechanisms including glomerular haemodynamics, tubular reabsorption of sodium, actions of renin-angiotensin system and natriuretic peptides[1]. Rare genetic variants that affect expression of molecules and pathways operating within the kidney lead to elevated BP in monogenic forms of hypertension[2]. Several common variants in genes associated with BP and/or susceptibility to hypertension are also believed to act through the renal mechanisms[3-7]. Two of these variants map to the same fibroblast growth factor 1 signalling cascade – a pathway increasingly recognised as an important player in cardiovascular system[6-7]. Indeed, common alleles of fibroblast growth factor 1 gene (FGF1) and its chaperone molecule gene (fibroblast growth factor binding molecule – FGFBP1) co-segregated with familial susceptibility to hypertension in our previous studies[6-7]. The central gene of this pathway – FGF1 – was up-regulated at both mRNA and protein level in the hypertensive kidney[6-7]. The recent large scale genetic analysis showed that genetic score composed of FGF1 signalling pathway single nucleotide polymorphisms (SNPs) provides a better explanation for variance in hypertension risk than the score calculated using a similar number of top variants identified by genome-wide association study[8]. Taken together, these data clearly suggest that FGF1 and its partner molecules play a role in genetic susceptibility to hypertension, possibly through kidney-related mechanisms. However, the exact biological cause of the association between FGF1 and BP regulation remains unknown.

Here we examined the association between a common SNP of FGF1 and BP in a meta-analysis of ≈15,000 individuals from 5 populations of white European ancestry. We then explored an effect of this SNP on expression of FGF1 mRNA in the largest to date analysis of 126 human kidneys collected in in the TRANScriptome of renaL humAn TissueE (TRANSLATE) Study. Through next-generation RNA-sequencing (RNA-seq) of human renal tissue we have investigated the network of most likely renal partner genes and transcripts of FGF1. Through further clinical association studies, *in silico* analyses and the investigations of biochemical read-outs of the most relevant partner molecules we

have identified a biologically plausible network of transcripts that can mediate renal FGF1-related BP effect.

Results

A common SNP of FGF1 (rs152524) is associated with blood pressure in the meta-analysis of 5 populations

The major demographic and clinical characteristics of 14364 individuals from 5 populations are given in Table 1. Distribution of rs152524 genotypes did not violate Hardy-Weinberg equilibrium[6] in either of the cohorts and the minor allele frequency of rs152524 in all studies was typical for white European population (Table S1). There was at least nominally significant association between rs152524 and SBP in 4 out of 5 studies. The meta-analysis of all individuals with available genotypic and phenotypic information revealed a significant association between clinic SBP and rs152524 – its each major allele copy increased SBP by ≈0.9 (±0.2) mmHg ($P=9.65x10^{-5}$) (Figure 1). The association between rs152524 and clinic DBP was directionally similar but the magnitude of the phenotypic effect of the SNP was smaller ($P=7.61x10^{-3}$) (Figure 1).

Major allele of rs152524 is associated with up-regulation of FGF1 mRNA in the human kidney

Clinical characteristics of 126 TRANSLATE Study individuals with informative genotype included in quantitative real-time PCR analysis of renal FGF1 are given in Table 1. rs152524 was associated with renal expression of total FGF1 – compared to the reference genotype (rare homozygous), carriers of one and two copies of major allele of rs152524 had 1.8 and 2.7-fold higher (respectively) levels of FGF1 mRNA in the kidney (P=0.009) (Figure 2).

Potential transcriptional activity of rs152524 and its statistical proxies – Roadmap Epigenomics and ENCODE analysis in silico

rs152524 maps to the segment of FGF1 intron 1 showing histone modifications in cells from adipose tissue, brain and skin indicating that the region may act as a transcriptional enhancer (Table S2). In addition, the rs152524-containing part of FGF1 intron 1 is a deoxyribonuclease I (DNase I)

hypersensitivity site (DHS), contains a HOXA5 transcription factor binding site and both alleles of rs152524 are predicted to show differential effect on binding of HOXA5. The rs152524 has at least five statistically similar (r^2>0.8) SNPs, of which 3 lie within regulatory enhancer regions identified in several human tissues (Table S2). The strength and quality of regulatory annotations for all proxy SNPs was lesser than that for rs152524 (Table S2). All detected proxies are located within the FGF1 gene but none of them map to any of four previously reported promoters in 5' region of FGF1[9-11]. No other known SNPs within a 1 Mb distance of rs152524 may account for the detected associations through linkage disequilibrium (LD) with rs152524 (Figure S1).

Increased expression of FGF1 and its three renal mRNA isoforms is associated with hypertension and higher blood pressure - next-generation RNA-sequencing of human kidney transcriptome

The available characteristics of individuals whose renal samples underwent next-generation RNA-seq are shown in Table S3. A total of 54043 mRNAs mapping to 18677 genes were expressed in 32 human kidneys from TRANSLATE Study. The RNA-seq identified 3 mRNA isoforms of FGF1 and revealed that FGF1-001 was the major transcript accounting for 78.5% of detectable renal FGF1 (Figure 2). The abundance of two other FGF1 transcripts (FGF1-003 and FGF-006) was lower at 10.3% and 11.2% (respectively) (Figure 2). The expression of all FGF1 transcripts showed a high level of linear inter-correlation (Table S4). The percentage abundance of FGF1 mRNA isoforms and their co-expression patterns were largely replicated in an independent collection of 70 apparently healthy renal tissues from The Cancer Genome Atlas (TCGA) project (Figure 2, Table S5).

Both FGF1 globally and its 3 mRNAs separately were ≈31-37% more abundant in hypertensive than normotensive kidneys from TRANSLATE Study (where appropriate phenotypic information was available) (Table S6). Consistently, the expression of total FGF1 and its 3 renal mRNA isoforms was associated with clinic BP in the expected direction (Table 2).

Renal FGF1 shows a very high level of co-expression with over 100 mRNAs in the human kidney

After correction for multiple testing (false discovery rate q<0.1%) a total of 747 mRNAs collapsed to 506 genes showed association with renal expression of FGF1 in TRANSLATE Study (Figure S2). A total of 126 non-FGF1 transcripts in 101 genes associated with FGF1 in TRANSLATE population replicated at a conservative FDR<0.1% in the TCGA (Figures 3,S3, Table S7). There was an excellent consistency in both average expression and the magnitude of association with FGF1 for those transcripts between the discovery and the replication population (Figure 2, Figure S4).

Renal transcripts co-expressed with FGF1 mRNA are associated with BP and have a strong relevance to human hypertension

Of 126 non-FGF1 mRNAs correlated with expression of FGF1 in kidneys, 71 and 63 showed at least nominal association with SBP and DBP, (respectively) in TRANSLATE Study (Table S8). This translates into 57 and 51 individual genes whose mRNAs are associated with SBP and DBP. Five genes (membrane metallo-endopeptidase gene - MME, protein tyrosine phosphatase, receptor type, O gene - PTPRO, renin gene - REN, solute carrier family 12 (sodium/chloride transporters), member 3 gene - SLC12A3 and WNK lysine deficient protein kinase 1 gene - WNK1) associated with BP in the study and tightly co-expressed with FGF1 had direct prior annotation to BP regulation either through Gene Ontology or by manual data mining (Table S9). The direction of association between the renal expression of these five genes and FGF1 abundance as well as BP was positive – the higher their expression, the higher renal abundance of FGF1 and the higher BP. *In silico* exploration of the existing datasets of renal expression profiling revealed that similar to FGF1, MME and PTPRO have the strongest enrichment for expression in the glomeruli (Table S10). Further comparative co-expression analysis revealed that hypertensive kidneys exhibit much stronger co-regulation between MME transcripts and FGF1 mRNA than normotensive ones (Figure 2).

Renal up-regulation of FGF1 mRNA is associated with lower circulating levels of natriuretic peptides

In 32 subjects whose kidney samples underwent next-generation RNA sequencing renal expression of FGF1 showed negative correlation with circulating levels of brain natriuretic peptide (BNP) (r=-

0.359, P=0.044). This association retained its statistical significance after adjustment for other clinical variables (β=-0.022, SE=0.009, P=0.023). Circulating levels of pro-atrial natriuretic peptide (proANP) showed the same direction of correlation (r=-0.287, P=0.111). Upon specified criteria, renal FGF1 mRNA showed a negative association with circulating proANP in multiple regression analysis (β=-0.018, SE=0.009, P=0.057). The direction of associations between serum concentrations of both natriuretic peptides and renal expression of MME mRNA was similar to that of FGF1 mRNA but the level of statistical significance of these associations was somewhat weaker in multiple regression analysis (P=0.083 and P=0.138 for proANP and BNP, respectively). FGF1 showed also positive association with natriuretic peptide receptor 3 (NPR3) at the renal expression level ($P=3.4x10^{-8}$).

Discussion

Our study has provided several important new insights into the association between FGF1 and BP. Firstly, we revealed that the common allelic variant of FGF1 previously associated with familial susceptibility to hypertension is also related to BP as a continuous quantitative trait in a large sample of individuals recruited primarily from the general populations. Secondly, we provided data suggestive of an effect of this variant on FGF1 mRNA in the kidney. Thirdly, through next-generation RNA-sequencing we have characterised the diversity of renal FGF1 mRNAs and quantified the extent of their over-expression in hypertensive kidney. Most importantly, through co-expression analysis we have identified the network of FGF1 partner transcripts and genes that may explain how up-regulation of FGF1 mRNA in the kidney could relate to BP elevation.

FGF1 was identified as a strong positional and potentially functional candidate gene for hypertension in our previous studies combining linkage analysis with fine mapping, rat-human synteny and direct sequencing[6,12]. Our subsequent family-based association analysis revealed that a major allele of common FGF1 SNP (rs152524) was transmitted from heterozygous parents to essentially hypertensive offspring more frequently than expected by chance[6]. The data presented here clearly demonstrate that rs152524 is also associated with both SBP and DBP as continuous traits and that the direction of allelic association with BP is concordant with that observed for hypertension. As expected

from a common SNP, the magnitude of the allelic effect on BP is small (≈0.9 mmHg increase in SBP per major allele copy), consistent with the findings on common variants associated with BP in large genetic meta-analyses[3]. However, in contrast to many genetic associations identified before, the relationship between rs152524 and BP has biologically meaningful functional context – a major allele of rs152524 not only correlated with BP elevation but also – an increase in renal expression of FGF1 mRNA. Further studies will be needed to confirm that rs152524 acts as *cis*-acting gene expression Quantitative Trait Locus (cis-eQTL) for FGF1 in the kidney. Our previous direct sequencing studies excluded common SNP within the coding sequences of FGF1 that could account for the detected associations by i.e. LD with rs152524[6]. The rs152524 maps to a putatively regulatory segment of FGF1 intron 1 in several tissues; the absence of kidneys in ENCODE and Roadmap Epigenomics did not permit us to explore potential regulatory role of rs152524 in tissue relevant to our study. It is tempting to surmise that the characterised region in intron 1 may operate as an enhancer and possibly, in concert with previously identified alternative promoters[9-11], contribute to transcriptional regulation of FGF1. Indeed, four different promoters in 5' region of FGF1 were reported to control its transcription into four different mRNA isoforms in cell/tissue-specific manner[9-11]. 5' regions of two FGF1 mRNA isoforms identified in our next-generation RNA-sequencing experiment as FGF1-003 and FGF1-006 best overlap with sequences regulated by promoter 1A whilst FGF1-001 – with promoter 1D[9-11]. FGF1 promoter 1A was reported as operational in the kidney and promoter 1D was identified as active in vascular smooth muscle cells (VSMC) and fibroblasts[11]. Both fibroblasts and VSMC are resident cells of the human kidney[13]. However, we should acknowledge the inherent limitation of selection of poly-adenylated RNA molecules in sample preparation – the use of this biochemistry is known to lead to 3' bias in RNA-sequencing experiments, thus fine-scale 5' promoter usage may be more challenging to resolve[14-15].

The kidney is a key organ for BP regulation and the site of highest FGF1 expression (http://www.proteinatlas.org/ENSG00000113578/tissue). FGF1 belongs to one of glomerulus-enriched genes[16-19] and both our and others' previous immunochemistry confirmed the exclusive expression of FGF1 within apparently normal human glomeruli[6,20]. A number of mRNAs (i.e. Nephrosis 1, congenital, Finnish type (nephrin) gene - NPHS1, Nephrosis 2, idiopathic, steroid-

resistant (podocin) gene - NPHS2) most tightly co-expressed with FGF1 in kidneys examined here are known as key structural constituents of glomerulus[21]. Taken together these data suggest that the main direct biological actions of FGF1 in the kidney occur in glomeruli. Given a diversity of biological processes that FGF1-co-expressed mRNAs map onto, we suspect that FGF1 may be involved in regulation of structural integrity of glomeruli and renal maintenance of metabolic homeostasis.

FGF1 is a strong mitogenic signalling molecule[22] but the mechanisms underlying its association with BP are not clear. Given that levels of mRNA/gene co-expression are good measures for activity of networks that control complex genetic mechanisms[23], we used renal next-generation RNA-seq data to identify FGF1 partner molecules and elucidate how increased expression of FGF1 in the kidney may link to BP regulation. Of FGF1 co-expressed mRNAs, those associated with SBP and/or DBP in the TRANSLATE Study and having direct prior annotation to BP regulation are the strongest putative mediators of FGF1-related BP effect. Indeed, four of these genes (REN, MME, WNK1 and SLC12A3) belong to key BP regulatory cascades and three (REN, MME and SLC12As) are direct targets for antihypertensive medications. MME encodes membrane metallo-endopeptidase (neutral endopeptidase or neprilysin) – an enzyme responsible for biochemical degradation of circulating peptides many of which promote vasodilation and/or natriuresis i.e. bradykinin, ANP and BNP[24]. Pharmacological inhibition of neprilysin leads to increased levels of natriuretic peptides and was proposed as a blood pressure lowering therapy[24]. Indeed, hypertension is increasingly recognised as a state of ANP and BNP deficiency[24]. Similar to FGF1, MME is highly expressed within renal glomeruli[25]. Renin is responsible for cleavage of angiotensin I from angiotensinogen - a rate-limiting step in activation of a fundamental pathway of BP regulation[26]. The products of WNK1 and SLC12A3 belong to a key cellular mechanism of sodium reabsorption in the distal convoluted tubule of the nephron and their up-regulation is a recognised renal mechanism of hypertension[27]. Unlike FGF1 and MME, REN is expressed primarily within juxto-glomerular apparatus and collecting duct whilst WNK1 and SLC12A3 are expressed in the distal nephron[26-27]. Taken together these data indicate that increased expression of FGF1 in the kidney correlated with up-regulation of MME, REN, SLC12A3 and WNK1 transcripts may contribute to BP elevation, potentially through heightened catabolism of

natriuretic peptides clearance/degradation (MME), activation of renin-angiotensin cascade (REN) and increased sodium reabsorption (SLC12A3 and WNK1). Furthermore, the direction of association between renal abundance of MME, REN, SLC12A3 and WNK1 mRNAs and BP in TRANSLATE Study (increased expression = higher BP) fits into their potential role as mediators of BP elevating effect of FGF1 overexpression. The correlation between decreased circulating levels of key substrates (natriuretic peptides) for MME and up-regulation of renal FGF1 further suggests that at least one of its identified renal co-expressions translates into biologically active mechanism measurable in the systemic circulation. One of the potential explanations for the somewhat weaker associations between natriuretic peptides and MME mRNA than those identified between both peptides and FGF1 mRNA is that increased renal expression of FGF1 mRNA correlates with up-regulation of another, MME-independent mechanism of natriuretic peptide catabolism in the kidney. Our data support this hypothesis - renal expression of FGF1 showed a positive association with NPR3 mRNA – an important clearance receptor for natriuretic peptides. In this context, increased expression of glomerular FGF1 may lead to a drop in levels of natriuretic peptides potentially through renal activation of their clearance/degradation pathway whilst increased expression of some other FGF1-co-expressed mRNAs in the kidney (i.e. REN, SLC12A3, WNK4) may simply indicate the activation of mechanisms secondary to the reduction in ANP/BNP circulating levels. To this end, lower levels of natriuretic peptides are synonymous with the reduction of their inhibitory effect on tubular sodium reabsorption and their weaker opposing effect on suppression of renin[28]. Indeed, our data showed that expression of MME mRNA correlates positively with abundance of REN, WNK1 and SLC12A3 mRNAs in kidneys from both TRANSLATE and TCGA cohorts (Figure 3 and Figure S3 – the most inner parts).

We should acknowledge that given the inherent limitations of our RNA-sequencing experiments, we cannot assign the direction to the identified co-expression patterns/associations and/or assess the extent to which they are primary (causal) or secondary (reactive). The association between FGF1 mRNA and its common SNP suggests that at least a proportion of renal increase of FGF1 mRNA is genetically regulated. It is also fair to point out that other FGF1-co-related transcripts and genes including several non-coding RNAs without prior linkage to BP regulation ontology terms may

mediate/contribute to the association between FGF1 and BP. We appreciate the limitations of RNA profiling of the kidney as the whole organ[17]. Indeed, cellular diversity of the kidney may lead to variation in transcriptome composition between different segments of the nephron[17]. Single cell transcriptomics will be helpful to refine the identified co-expression patterns. Finally, we appreciate that our study is based primarily on expression analysis of FGF1 mRNA. Further experiments at the protein level will be necessary to confirm our findings.

Within the interpretational limitations discussed above our data suggest that the expression of FGF1 mRNA in the human kidney is at least partly genetically controlled and that FGF1 maps onto several major renal pathways of BP regulation. Our study also illustrates the potential of next-generation RNA-seq in relevant human tissues to provide insights into mechanisms underlying associations between candidate genes and complex phenotypes.

Concise methods

Populations

Five populations (Silesian Cardiovascular Study – SCS[29], Victorian Family Heart Study – VFHS[30] Prevention of Renal and Vascular End-stage Disease Study – PREVEND[31-32], Genetic Regulation of Arterial Pressure of Humans in the Community Study – GRAPHIC[5,33] and Young Men Cardiovascular Association Study – YMCA[34]) with relevant genetic (directly genotyped rs152524 SNP) and phenotypic (clinic blood pressure) information were included in the genetic association analysis. All individuals were of white European ethnicity.

TRANSLATE Study provided human kidney samples for quantitative real-time PCR analysis of FGF1 (n=133) and the discovery phase of the next-generation RNA-sequencing analysis (n=32). In brief, the TRANSLATE Study recruited individuals of white European ancestry eligible for unilateral elective nephrectomy because of non-invasive renal cancer in three reference centres[7]. Small fragments of kidney tissues were collected from healthy (unaffected by cancer) pole of the kidney and immersed in RNAlater (Ambion, Austin, TX) immediately after nephrectomy. Information on an

additional set of 70 human kidneys characterised by RNA-sequencing used in the replication phase of next-generation RNA-sequencing analysis was obtained from TCGA database[35]. The tissue material was secured from tissue unaffected by cancer (healthy control) after renal nephrectomy due to cancer[36].

The Bioethical Committees have approved the studies and all subjects have given written informed consent for participation. The studies adhered to the Declaration of Helsinki. Information on the recruitment and phenotyping of each individual study is summarised in the Supplementary material.

DNA analysis

DNA was extracted from peripheral blood leukocytes according to the previously described protocols[5,12,29,34]. In all populations, the SNP of interest was genotyped directly – in GRAPHIC by MALDI-TOF mass spectrometry (MassARRAY system, Sequenom Inc.) and in other cohorts - by TaqMan *(*ABI PRISM 7900HT Sequence Detection System - Applied Biosystems).

RNA analysis

RNA was extracted from kidneys using previously reported protocols[6]. The measurement of total FGF1 mRNA in 126 TRANSLATE Study kidneys with informative rs152524 genotype was conducted by SYBR® Green-based real-time quantitative PCR on Eppendorf Realplex PCR equipment (Eppendorf, Sydney, Australia). The discovery next-generation RNA-sequencing experiment in 32 TRANSLATE kidneys was conducted on Illumina HiSeq™ 2000 sequencer (Illumina, San Diego, California, USA) using 100-bp paired-end reads. 70 TCGA kidneys were also sequenced on an Illumina HiSeq 2000 and raw (level 1) 50 bp reads were downloaded from CGHub (https://cghub.ucsc.edu/).

Biochemical studies

Serum levels of BNP and proANP (1-98) were measured in TRANSLATE individuals who underwent renal RNA-seq by direct chemiluminescence on Centaur XP platform (SIEMENS, Munich, Germany)

and enzyme immunoassay (BioMedica, Wien, Austria) on Asys Hitech Expert 96 (BIOGENET), respectively.

Statistical and bioinformatic analysis

The analysis of association between rs152524 and treatment-corrected clinic blood pressures was conducted by multiple linear regression after adjustment for additional clinical variables and under additive model of inheritance in each cohort individually and then jointly in fixed-effects inverse variance meta-analysis. The effect of rs152524 on quantitative PCR expression measures of FGF1 mRNA was examined under the same model of inheritance and after adjustment for demographic and technical variables in linear regression. *In silico* analysis of biological activity of rs152524 was conducted using data from Roadmap Epigenomics[37] and ENCODE[38]. Circulating levels of BNP and proANP underwent log- and inverse transformation prior to association studies. Their association with RNA-seq measure of FGF1 expression in the kidney were examined by Pearson's correlation and multiple linear regression after adjustment for clinical and demographic parameters.

The renal RNA sequencing data were processed through the Tuxedo workflow and the ultimate abundance of each mRNA was calculated in $\log_2$ transcripts per million (TPM) units. Baseline co-expression analysis with FGF1 was conducted for all renal mRNAs (individually and after collapsing to genes) by LIMMA with adjustment for available clinical and demographic variables in both TRANSLATE Study and TCGA. The correction for multiple testing calculated by false discovery rate was set at 0.1% in both datasets.

The extended version of the statistical and bioinformatics analysis is available in Supplementary material.

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Statement of competing financial interests

No competing interests.

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Table 1. Demographic and clinical characteristics of populations.

Variable	SCS	VFHS	PREVEND	GRAPHIC	YMCA	TRANSLATE
number of subjects	764	2755	7687	1987	1151	126
M/F	439/325	1334/1421	3784/3903	1005/982	1151/0	71/55
Age (years)	55.2±11.8	39.7±15.8	49.5±12.8	39.3±14.5	19.1±3.6	61.1±10.5
Body mass index (kg/m^2)	27.5±4.2	25.1±4.1	26.1±4.2	26.1±4.6	22.8±3.0	27.6±4.4
Clinic SBP (mmHg)	131.3±19.1	123.3±15.2	129.3±20.3	127.1±17.8	118.0±13.1	136.4±13.5
Clinic DBP (mmHg)	74.9±11.0	75.7±10.7	75.6±11.2	79.2±11.0	74.2±7.9	83.2±8.0
Hypertension (%)	549 (71.9)	515 (18.6)	2647 (34.4)	571 (28.7)	120 (10.4)	85 (67.5)
Antihypertensive treatment (%)	451 (59.0)	222 (8.1)	1219 (15.9)	134 (6.7)	18 (1.6)	74 (58.7)

Data are counts and percentages or means and standard deviations, SBP – systolic blood pressure, DBP – diastolic blood pressure, SCS – Silesian Cardiovascular Study; VFHS – Victorian Family Heart Study, PREVEND – Prevention of Renal and Vascular End-stage Disease Study, GRAPHIC – Genetic Regulation of Arterial Pressure of Humans in the Community Study, YMCA – Young Men Cardiovascular Association Study, TRANSLATE – TRANScriptome of renaL humAn TissueE Study

Table 2. Association between FGF1, its renal mRNAs and blood pressure in TRANSLATE Study – next-generation RNA-sequencing analysis of human kidneys.

mRNA	SBP		DBP	
	β-coefficient (SE)	P-value	β-coefficient (SE)	P-value
FGF1 total	0.85±0.35	0.021	1.15±0.57	0.053
FGF1-001	0.91±0.36	0.016	1.21±0.58	0.046
FGF1-003	0.091±0.034	0.0083	0.13±0.06	0.028
FGF1-006	0.13±0.05	0.016	0.14±0.08	0.092

Data are β-coefficients, standard errors (SE) and level of statistical significance (P-value) from linear regression models wherby clinic blood pressure (adjusted for treatment) was a dependent variable, and mRNA expression level (in TPM units) of total FGF1 or its individual mRNAs, age, sex and body mass index were independent parameters

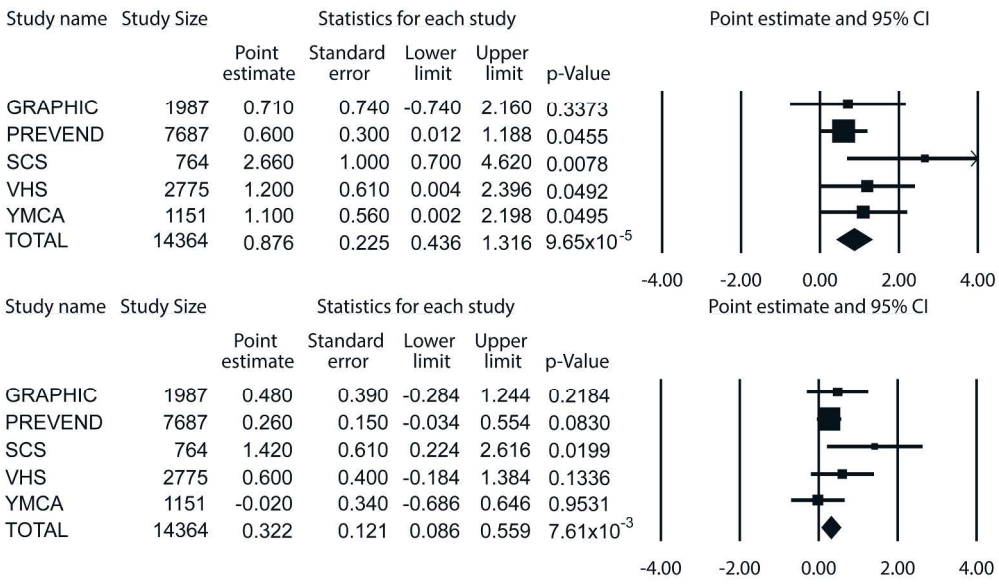

Figure 1. Association between single nucleotide polymorphism of FGF1 (rs152524) and blood pressure in 14344 individuals from 5 populations. Data are expressed as β-coefficients (Point estimate), with standard errors and lower/upper limits of the confidence intervals together with respective levels of statistical significance (P-value); the data come from regression analysis of systolic blood pressure (upper panel) or diastolic blood pressure (lower panel) as independent variables together with rs152524 genotype and demographic phenotypes as dependent parameters; major allele or rs152524 (A) is a reference allele.
246x141mm (300 x 300 DPI)

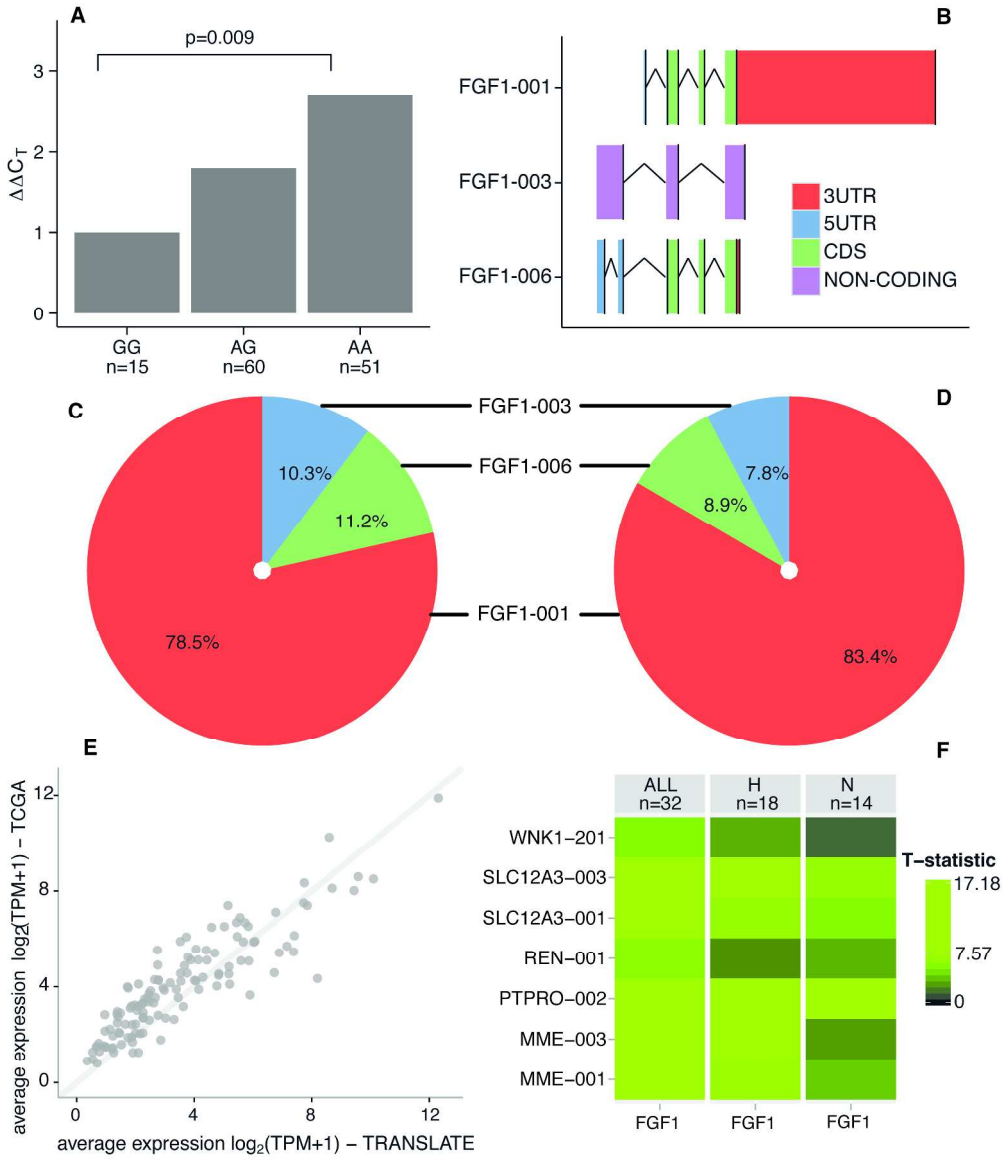

Figure 2. Renal expression of FGF1, its main mRNA isoforms and partner mRNAs in the human kidney. A. Relative fold differences in expression of total FGF1 mRNA between rs152524 genotypes – quantitative real-time PCR analysis of 126 human kidneys, P – level of statistical significance, n – number of individuals in each genotype group; B. The schematic structure of 3 renal FGF1 mRNA isoforms, 3UTR – 3′ untranslated region, 5UTR – 5′ untranslated region, CDS – coding sequence, FGF1-001 and FGF1-006 contain the same set of translated exons but differ in the structure of both 5′ and 3′ regions, FGF1-003 is a non-coding mRNA with a retained intron; CD. Percentage abundance of FGF1 mRNA isoforms in relation to total renal FGF1 mRNA in TRANScriptome of renaL humAn TissueE (TRANSLATE) Study (C) and The Cancer Genome Atlas (TCGA) resource (D); E. A total of 126 mRNAs co-expressed with FGF1 in the human kidney – consistency in the average expression between the discovery population (TRANSLATE Study) and the replication resource (TCGA), log2 TPM+1 – unit of expression from next-generation RNA-sequencing; F. Renal co-expression between FGF1 and 7 transcripts in 5 genes with highest relevance to blood pressure regulation – comparison of hypertensive (H) and normotensive (N) kidneys from the next-generation RNA-sequencing experiment, t-statistic - the magnitude of pair-wise co-expression calculated from a linear regression and expressed by

colour intensity (from black – least co-expressed to green – most co-expressed).
285x336mm (300 x 300 DPI)

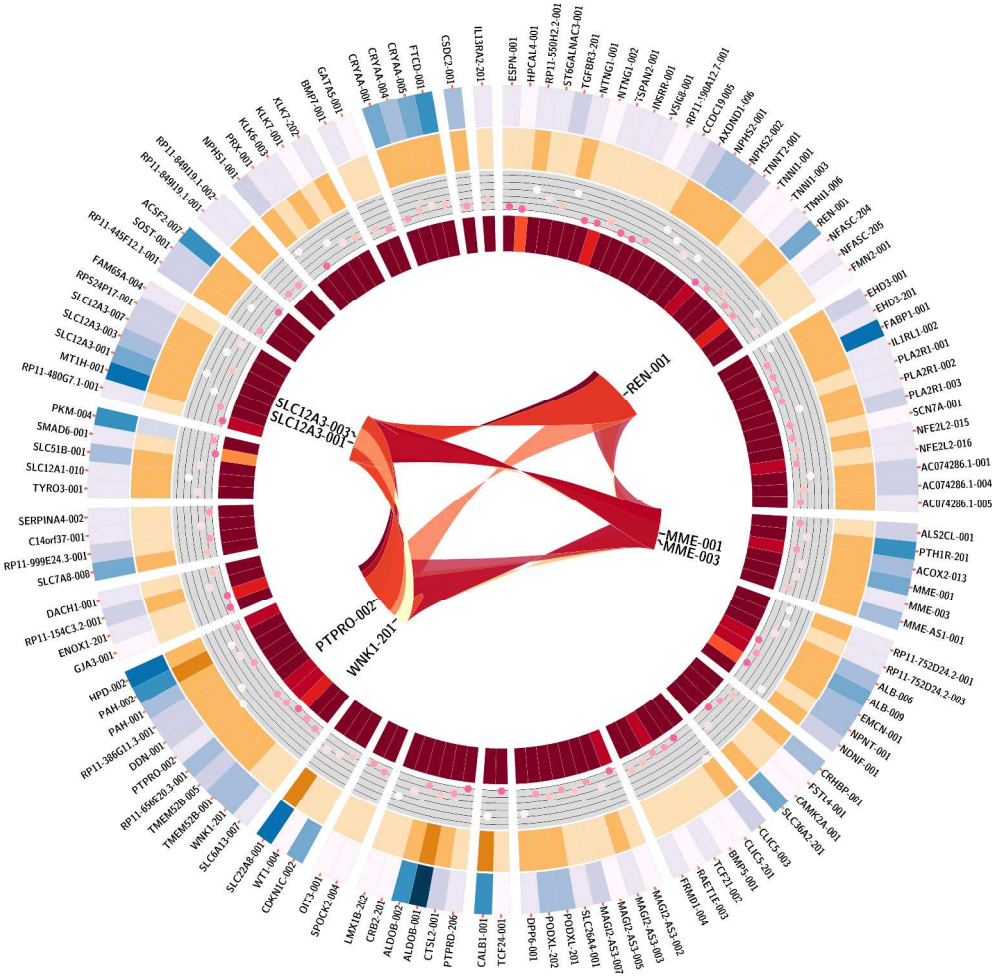

Figure 3. Replicated partner mRNAs of FGF1 in the kidney – next-generation RNA-sequencing in TRANScriptome of renaL humAn TissueE (TRANSLATE) Study. Replicated mRNAs – mRNAs associated with expression of FGF1 in both the TRANSLATE Study and The Cancer Genome Atlas resource; outermost circle – symbols of 126 mRNAs ordered in circular manner; first circle below – level of renal expression for each replicated partner mRNA (in log2 TPM+1 values) whereby white – lowest expression and navy blue – highest expression; second circle below – level of co-expression (measured as β-coefficient from linear regression) between each partner mRNA and FGF1 mRNA whereby dark brown – strong positive co-expression and beige – weak positive co-expression; third circle below – level of statistical significance (measured as –log10 P-value from linear regression) for co-expression between each partner mRNA and FGF1 mRNA, whereby white – strong statistical significance and pink – weak statistical significance; most inner circle – level of connectivity of partner mRNAs whereby dark red – highly connective mRNAs, orange – mRNAs with low connectivity; inside – co-expression between selected mRNAs relevant to blood pressure regulation. 1322x1322mm (96 x 96 DPI)

Renal mechanisms of association between FGF1 and blood pressure

Supplement

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Extended methods section

Populations

Silesian Cardiovascular Study (SCS)

SCS is a cohort of 1138 individuals (213 families with 703 subjects and 435 subjects with no families) recruited in Southern Poland through index patients (in families – parents) with high cardiovascular risk (defined as hypertension and/or coronary artery disease and/or clustering of multiple cardiovascular risk factors), as reported before (1). Each subject underwent thorough clinical, anthropometric, and biochemical phenotyping (1). Oscillometric blood pressure (BP) measurements were conducted in triplicate and 3 readings were averaged in to estimate the final systolic BP (SBP) and diastolic BP (DBP). All biologically unrelated individuals (807 subjects) from the previous FGF pathway study (2) were included in this analysis. A total of 764 individuals with complete genotype and phenotype information were included in the association analysis.

The Victorian Family Heart Study (VFHS)

This cohort of families of white European ancestry was recruited from the general population of Victoria (Australia) (3). Each of 783 nuclear families consisted of both parents (aged 40-70 years) and at least one natural offspring (aged 18-30 years). Phenotyping included taking medical history, clinical examination, and blood biochemistry (3). Supine BP was measured after resting (for at least 10 minutes) using standard sphygmomanometry. Of three BP measurements taken, the last two were recorded and averaged in calculation of the final SBP and DBP values. A total of 2755 individuals with full genotypic and phenotypic information were included in the genetic association analysis.

Prevention of Renal and Vascular End-stage Disease (PREVEND) Study

PREVEND cohort consists of biologically unrelated individuals from the adult (age range: 28-75 years) general population of Groningen (the Netherlands) unselected for hypertension but enriched for microalbuminuria, as reported before (4-5). In brief, 40856 subjects who responded to invitation to participate were screened for microalbuminuria. A total of 7768 individuals with urinary albumin concentration of ≥10mg/L together with 3395 subjects randomly selected from amongst those who had urinary albumin concentration of <10 mg/L were invited for further investigations in an outpatient clinic (4-5). A total of 8592 subjects who completed the screening program were included in the study group. Of those individuals of non-European ancestry and those with missing DNA were excluded prior to genotyping. BP was measured automatically in a supine position using automatic method (Dinamap XL Model 9300) during 2 clinic appointments. The mean of the last two measurements taken at the two visits was used in calculation of the final SBP and DBP values (4-5). Complete genotype and phenotype information was available for 7687 individuals.

Genetic Regulation of Arterial Pressure of Humans in the Community (GRAPHIC) Study

The details of recruitment, phenotyping and general clinical characteristics of GRAPHIC subjects were reported before (6-7). In brief, white British nuclear families with both parents (aged 40-60 years) and two adult offspring (aged ≥18 years) were identified through general practices in Leicestershire (UK) and invited to participate. There were no exclusion criteria apart from a known history of renal disease. A total of 2037 subjects from 520 nuclear families underwent detailed clinical phenotyping including medical history, anthropometric measurements, extensive blood biochemistry, along with BP measurements (7). Clinic BP was measured in triplicate using automatic device (Omron HEM-705CP monitors) and the mean of the second and third readings was used in calculation of the final values of SBP and DBP. A total of 1987 individuals with complete genetic and phenotypic data were included in the association analysis.

Young Men Cardiovascular Association (YMCA) Study

This cohort of 1157 young (average age: 19 years) men was recruited in Silesia (Southern Poland) (8). In brief, apparently healthy men of white European ancestry with no prior history of cardiovascular disease (apart from hypertension) were recruited from secondary schools of Silesia. As a part of phenotyping demographic and clinical information was collected using standardised coded questionnaires prior to anthropometric measurements and biochemical analyses (8). Blood pressure

was measured in a sitting position using calibrated mercury sphygmomanometer (8). Three readings were averaged in calculation of the final SBP and DBP values (8-9). A total of 1151 men were fully informative in the genetic association analysis.

TRANScriptome of renaL humAn TissueE (TRANSLATE) Study

TRANLSATE Study provided human kidney samples both for quantitative real-time PCR analysis of FGF1 (n=133) and the discovery phase of the next-generation RNA-sequencing analysis (n=32).

Human kidney samples from the TRANSLATE Study were collected after surgery in 133 patients who underwent elective unilateral nephrectomy because of non-invasive renal cancer in one of three nephrology-urology centres [Silesian Renal Tissue Bank (2,10), TRANSLATE P (recruitment conducted in Western Poland) and TRANSLATE Z (recruitment conducted in Southern Poland)]. Small fragments of kidney tissues were collected from healthy (unaffected by cancer) pole of the kidney and immersed in RNAlater (Ambion, Austin, TX) immediately after nephrectomy (2). Each subject underwent standardised clinical phenotyping, including personal history (through anonymous coded questionnaires), anthropometry (weight, height) and triplicate measurements of BP using a mercury sphygmomanometer (in a subset of samples – Silesian Renal Tissue Bank) (2,10) or automatic digital BP monitoring (in the rest of the patients). Diagnosis of hypertension was based on BP values ≥140/90 mmHg (measured on at least two separate occasions) and/or being on pharmacological anti-hypertensive treatment.

All recruited individuals were of white European ancestry.

RNA analysis

Renal expression of FGF1 mRNA - SYBR® Green-based real-time quantitative PCR

The sequences of human FGF1 mRNA was obtained from Ensembl database. Primer3Plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) and PrimerQuest (https://www.idtdna.com/Scitools/Applications/Primerquest/Default.aspx) were used to design primers for total FGF1 - the primers were designed based on the sequence of the first coding exon of FGF1 and capture all other known transcripts. Quantitative real-time PCR (qPCR) of renal samples from TRANSLATE Study was performed on Eppendorf Realplex PCR equipment (Eppendorf, Sydney, Australia). The experiments were conducted using 5 µl SensiMix SYBR No-ROX (Bioline, Sydney, Australia), 0.25 µl of each specific primer (GeneWorks, Adelaide, South Australia), 1 µl of renal cDNA (100 ng) and H_2O to a final 10 µl volume. qPCR was carried out over 45 cycles of 95ºC for 15 sec, 57ºC for 15 sec, and 72ºC for 15 sec. The relative level of target gene expression was normalized to 18s ribosomal RNA.

Sequencing library preparation - TRANSLATE Study

Sequencing libraries were generated from 1 µg of total RNA for each sample and by following the Illumina TruSeq RNA sample preparation guide version 2 protocol. This protocol included poly-A transcript selection (via oligo-dT beads) and random hexamer primed first strand cDNA synthesis.

Statistical and bioinformatics analysis

Genetic association analysis

The concordance of rs152524 genotypes distribution with Hardy-Weinberg equilibrium was examined using a χ^2 test under the previously used threshold of $P<0.01$ (2,10).

Prior to association analysis, a correction for BP lowering effect of antihypertensive medication was applied - in all patients on pharmacological treatment a constant of 15 mmHg and 10 mmHg was added to measured SBP and DBP (respectively), similar to the algorithm used by International Consortium for Blood Pressure Genome-Wide Association Studies (9).

The analysis of association between BP and rs152524 in biologically unrelated subjects (SCS, PREVEND, YMCA) was conducted by linear regression under additive model of inheritance (coded as 0, 1 or 2 major allele copies). The regression models were constructed in PLINK and adjusted for age and sex (11). In VFHS families the association analysis was examined under the same model of inheritance using the PLINK family-based test for quantitative traits (QFAM) (12). The QFAM regressed age, age^2 and sex-adjusted SBP and DBP on rs152524 genotype and corrected for family

structure using adaptive permutations (12). The association analysis in the GRAPHIC cohort was carried out by GEMMA software that uses Mixed Model Association algorithm for a standard linear mixed model and some of its close relatives (13). GEMMA fitted linear mixed models (LMM) for association tests of rs152524 with SBP and DBP (accounting for family structure) In GRAPHIC Study. The analysis was also adjusted for age, age^2 and sex.
In all models β-coefficients along with the respective standard errors (SE) were calculated per each major allele copy of rs152524. Inverse variance weighted averages of β-coefficients and SE from all populations were pooled together in fixed effects meta-analysis. A combined effect size estimates and respective p-values were calculated for the effective sample of 14364 individuals under additive model of inheritance using METAL software (http://www.sph.umich.edu/csg/abecasis/metal/index.html). The between-study heterogeneity was computed using χ^2-test.

rs152524 – renal FGF1 mRNA association analysis
The compatibility of rs152524 genotypes distribution with Hardy–Weinberg Equilibrium was tested by χ^2 test. The effect of rs152524 on renal expression of total FGF1 mRNA was examined by regressing the qPCR-derived measure on rs152524 genotype (coded as 0, 1 or 2 major allele copies). The analysis was conducted by stepwise regression with clinical (age, sex, body mass index – BMI, hypertension) and technical (cohort origin, qPCR experiment) variables entered into the model under the criteria of F-statistic (Probability-of-F-to-enter ≤0.15, Probability-of-F-to-remove ≥0.2). Relative differences in expression of total FGF1 between rs152524 genotypes were calculated using $2^{-\Delta\Delta C_T}$ formula (14) whereby ΔC_T were residuals for qPCR-derived total FGF1 measures (delta cycle thresholds) from stepwise linear regression. Rare homozygous genotype was used as a baseline reference. A total of 126 samples were fully informative in genetic-mRNA association analysis.

rs152524 – regulatory analysis *in silico*
We investigated the regulatory potential of rs152524 single nucleotide polymorphism (SNP) and its statistical proxies [SNPs in high linkage disequilibrium coefficient (r^2>0.8) with rs152524]. We queried HaploReg v3, a tool that identifies SNPs and their proxies that overlap with regulatory regions, for all SNPs showing r^2-coefficient of at least 0.8 with rs152524. The information for transcriptional enhancer regions mapping to FGF1 was obtained from Roadmap Epigenomics (15) and ENCODE (16) database on patterns of histone modification, DNase I hypersensitive sites and transcription factor binding motifs.

Next-generation RNA-sequencing - TRANSLATE and The Cancer Genome Atlas (TCGA)
A total of 32 human kidney cDNA libraries from the TRANSLATE study were sequenced on an Illumina HiSeq 2000 using 100bp pair-end reads. This resulted in 4.4Gb (±0.48) of mapped sequence data per sample. Given an estimated expressed renal transcriptome size of 61.2Mb (see filtering below), the average coverage across the transcriptome is 73x.

Raw reads were quality checked using FastQC (17); post-alignment, the squared coefficient of variation and expression dispersion estimates were assessed using CummeRBund. Reads were filtered and aligned by TopHat v2.0.1 to the Ensembl GRCh37 reference genome using Ensembl v70 transcript annotations. Cuffdiff v2.1.1 was used to quantify the Ensembl v70 reference transcriptome. An assessment of background gene expression noise in the dataset was determined using repeat quantification of Ensembl gene structures randomly moved to intergenic locations throughout the genome. These quantifications were used to determine a lower cutoff of 0.128 FPKM which accounts for 95% of all non-reference genic expression across the dataset. All transcripts expressed above the lower cutoff in 50% or more of the samples were analysed further. Cuffdiff quantifications were converted into transcripts per million (TPM) units and then $\log_2$ transformed after adding a constant of 1 (i.e. A TPM of 0 stays at that value after log transformation). Logged TPM values were then adjusted using 5 hidden factors defined by probabilistic estimation of expression residuals (PEER), using the approach defined earlier (18-19).

TCGA sample fastq files were downloaded from CGHub (under agreement 26966-1). The TCGA samples were sequenced using 50bp paired end reads on an Illuina HiSeq 2000. The raw reads were aligned, and transcript expression was quantified, in the same manner as for TRANSLATE. TCGA samples were sequenced to greater depth, with 6.8Gb (±1.5Gb) of mapped sequence data per sample giving an average coverage across the transcriptome of 111x (assuming a 61.2Mb transcriptome).

Linear correlations between mRNA isoforms of FGF1 expressed in the kidney were examined by Pearson's linear correlation. The analysis of co-expression between FGF1 mRNA and renal transcripts was conducted using multiple linear regression models whereby individual transcripts were independent variables and FGF1 mRNA abundance (expressed in TPM units), age, sex and BMI – dependent parameters. This analysis was followed by collapsing significantly associated mRNA isoforms into genes they originate from. The obtained regression coefficients with the respective SE and levels of statistical significance were used as measures of co-expression between FGF1 and other mRNA. The correction for multiple testing was applied by estimating the false discovery rate (FDR). We used an FDR lower threshold of q=0.001 (0.1%) for detection of statistically significant co-expressed transcripts, genes and collapsed genes. Circle plots were produced using circos (20) to visualise levels of co-expression between FGF1 and correlated transcripts.

Differences in renal expression of total FGF1 mRNA and its mRNA isoforms between hypertensive and normotensive kidneys from TRANSLATE Study were calculated by linear regression with mRNA expression measures (expressed in TPM) as the dependant variable and age, sex, BMI and hypertensive status as independent parameters. Analysis of association between mRNAs and BP in the TRANSLATE Study was conducted by the linear regression with SBP/DBP as dependent variable and individual mRNAs (or genes where appropriate) expression measures obtained from next generation RNA-sequencing, age, sex and BMI as independent parameters.

Biochemical analysis

Serum levels of natriuretic peptides (proANP and BNP) measured in 32 individuals included in RNA-seq were normalised by inverse transformation prior to further association studies. Renal expression measures of FGF1 mRNA were obtained from next generation RNA-seq. Analysis of association between natriuretic peptides and FGF1 expression in the kidney was conducted by stepwise linear regression equations whereby natriuretic peptides levels were independent phenotypes and FGF1 mRNA, age, sex, and BMI – independent variables. The independent parameters were entered in the regression models based on F-statistic criterion (Probability-of-F-to-enter ≤0.25, Probability-of-F-to-remove ≥0.30).

Supplementary Tables

Table S1. Allele frequencies of rs152524 single nucleotide polymorphism in FGF1.

Study	Minor allele frequency (G)	Major allele frequency (A)
SCS	0.45	0.55
VFHS	0.43	0.57
PREVEND	0.45	0.55
GRAPHIC	0.43	0.57
YMCA	0.45	0.55
TRANSLATE	0.36	0.64

SCS – Silesian Cardiovascular Study, VFHS – Victorian Family Heart Study, PREVEND – Prevention of Renal and Vascular End-stage Disease Study, GRAPHIC – Genetic Regulation of Arterial Pressure of Humans in the Community Study, YMCA – Young Men Cardiovascular Association Study, TRANSLATE – TRANScriptome of renaL humAn TissueE Study; in family-based studies allele frequencies were calculated based on the data from parental generation

Table S2. Regulatory regions overlapping with rs152524 and its proxies in Roadmap Epigenomics and ENCODE.

SNP	r^2	Tissue/Cell type	Regulatory region type	Data source
rs152524	-	adipose derived mesenchymal stem cell cultured cells	weak enhancer	Roadmap Epigenomics
rs152524	-	brain angular gyrus	enhancer-like transcribed region	Roadmap Epigenomics
rs152524	-	imr90 cell line	active enhancer	Roadmap Epigenomics
rs152524	-	hesc derived cd56+ mesoderm cultured cells	weak enhancer	Roadmap Epigenomics
rs152524	-	penis foreskin fibroblast primary cells.donor skin02	transcription enhancer-like	Roadmap Epigenomics
rs152524	-	brain cingulate gyrus	transcription enhancer-like	Roadmap Epigenomics
rs152524	-	fetal muscle trunk	DNase I hypersensitive site	Roadmap Epigenomics
rs152524	-	fetal muscle leg	DNase I hypersensitive site	Roadmap Epigenomics
rs152524	-	T cell (leukaemia line)	Enhancer	Ensembl VEP
rs152524	-	mammary epithelial cells	Enhancer	Ensembl VEP
rs152524	-	skeletal muscle myoblasts	Enhancer	Ensembl VEP
rs152524	-	fetal lung fibroblasts	Enhancer	Ensembl VEP
rs152524	-	astrocytes	Enhancer	Ensembl VEP
rs152524	-	adult dermal fibroblasts	Enhancer	Ensembl VEP
rs152524	-	epidermal keratinocytes	Enhancer	Ensembl VEP
rs152524	-	lung fibroblasts	Enhancer	Ensembl VEP
rs152524	-	osteoblasts	Enhancer	Ensembl VEP
rs34012	0.92	fetal brain	weak enhancer	Roadmap Epigenomics
rs34012	0.92	fetal brain	DNase I hypersensitive site	Roadmap Epigenomics
rs249925	0.85	left ventricle	active enhancer	Roadmap Epigenomics
rs249925	0.85	chondrocytes from bone marrow derived mesenchymal stem cell cultured cells	weak enhancer	Roadmap Epigenomics
rs249925	0.85	stomach mucosa	weak enhancer	Roadmap Epigenomics
rs34005	0.8	mesenchymal stem cell derived adipocyte cultured cells	weak enhancer	Roadmap Epigenomics
rs34005	0.8	adipose derived mesenchymal stem cell cultured cells	weak enhancer	Roadmap Epigenomics
rs34005	0.8	muscle satellite cultured cells	active enhancer	Roadmap Epigenomics
rs34005	0.8	penis foreskin fibroblast primary cells	weak enhancer	Roadmap Epigenomics

rs34005	0.8	hesc derived cd184+ endoderm cultured cells	weak enhancer	Roadmap Epigenomics
rs34005	0.8	brain cingulate gyrus	active enhancer	Roadmap Epigenomics
rs34005	0.8	pancreatic islets	weak enhancer	Roadmap Epigenomics
rs34005	0.8	brain substantia nigra	weak enhancer	Roadmap Epigenomics
rs34005	0.8	penis foreskin melanocyte primary cells	weak enhancer	Roadmap Epigenomics
rs34005	0.8	mammary epithelial cells	weak enhancer	ENCODE
rs34005	0.8	lung fibroblasts	weak enhancer	ENCODE

SNP – single nucleotide polymorphism, r^2 – measure of linkage disequillibrium with rs152524 in CEU population, VEP – Variant Effect Predictor

Table S3. Demographic and clinical characteristics of individuals from TRANSLATE Study and TCGA resource included in next generation RNA-sequencing experiment.

Variable	TRANSLATE	TCGA
Number of subjects	32	70
M/F	19/13	51/19
Age (years)	59.0±7.6	62.9±12.0
Body mass index (kg/m^2)	26.9±4.4	N/A
Clinic SBP (mmHg)	140.1±15.4	N/A
Clinic DBP (mmHg)	88.0±7.7	N/A
Hypertension (%)	18 (50.0)	N/A
Antihypertensive treatment (%)	14 (43.8)	N/A

Data are counts and percentages or means and standard deviations, SBP – systolic blood pressure, DBP – diastolic blood pressure, TRANSLATE – TRANScriptome of renaL humAn TissueE Study, TCGA – Tissue Cancer Genome Atlas, N/A – not available

Table S4. Linear correlation in renal expression of FGF1 mRNA isoforms in TRANSLATE Study – next-generation RNA-sequencing analysis.

	FGF1-001	FGF1-003	FGF1-006
FGF1 total	r=0.95, $P<2.2x10^{-16}$	r=0.97, $P<2.2x10^{-16}$	r=0.95, $P<2.2x10^{-16}$
FGF1-001	-	r=0.86, $P=4.6x10^{-11}$	r=0.82, $P=3.3x10^{-9}$
FGF1-003	-	-	r=0.94, $P<2.2x10^{-16}$

Data are Pearson's correlation coefficient (r) and levels of statistical significance (P-values), TRANSLATE – TRANScriptome of renaL humAn TissueE Study

Table S5. Linear correlation in expression of FGF1 mRNA isoforms in TCGA – next-generation RNA-sequencing analysis.

	FGF1-001	FGF1-003	FGF1-006
FGF1 total	$r=0.99$, $P<2.2x10^{-16}$	$r=0.96$, $P<2.2x10^{-16}$	$r=0.96$, $P<2.2x10^{-16}$
FGF1-001	-	$r=0.92$, $P<2.2x10^{-16}$	$r=0.93$, $P<2.2x10^{-16}$
FGF1-003	-	-	$r=0.85$, $P<2.2x10^{-16}$

Data are Pearson's correlation coefficient (r) and levels of statistical significance (P-values), TCGA – Tissue Cancer Genome Atlas

Table S6. Association between FGF1, its renal mRNA isoforms and hypertension in TRANSLATE Study – next-generation RNA-sequencing analysis of the human kidneys.

	All subjects	Normotension	Hypertension	Mean crude excess	P-value
FGF1 total	56.6±7.5	48.5±10.1	63.8±11.1	31.5%	0.041
FGF1-001	47.6±6.2	40.9±8.5	53.6±9.0	31.2%	0.042
FGF1-003	4.1±0.6	3.4±0.6	4.7±1.0	36.7%	0.023
FGF1-006	4.9±0.8	4.2±1.1	5.5±1.3	31%	0.099

Data are means and standard errors for expression values from next generation RNA-sequencing analysis (in TPM units); mean crude excess – average expression excess in hypertension, P-value – level of statistical significance for comparison of hypertension and normotension from a linear regression model adjusted for age, sex and body mass index, TRANSLATE – TRANScriptome of renaL humAn TissueE Study

Table S7. Genes whose mRNAs are associated with renal expression of FGF1 in both TRANSLATE and TCGA Studies.

No.	Ensembl gene ID	Gene Symbol	Gene product name
1	ENSG00000213963	AC074286.1	Uncharacterized protein
2	ENSG00000168306	ACOX2	Acyl-CoA oxidase 2, branched chain
3	ENSG00000167107	ACSF2	Acyl-CoA synthetase family member 2
4	ENSG00000163631	ALB	Albumin
5	ENSG00000136872	ALDOB	Aldolase B, fructose-bisphosphate
6	ENSG00000178038	ALS2CL	ALS2 C-terminal like
7	ENSG00000162779	AXDND1	Axonemal dynein light chain domain containing 1
8	ENSG00000112175	BMP5	Bone morphogenetic protein 5
9	ENSG00000101144	BMP7	Bone morphogenetic protein 7
10	ENSG00000139971	C14orf37	Chromosome 14 open reading frame 37
11	ENSG00000104327	CALB1	Calbindin 1, 28kDa
12	ENSG00000070808	CAMK2A	Calcium/calmodulin-dependent protein kinase II alpha
13	ENSG00000213085	CCDC19	Coiled-coil domain containing 19
14	ENSG00000129757	CDKN1C	Cyclin-dependent kinase inhibitor 1C (p57, Kip2)
15	ENSG00000112782	CLIC5	Chloride intracellular channel 5
16	ENSG00000148204	CRB2	Crumbs homolog 2 (Drosophila)
17	ENSG00000145708	CRHBP	Corticotropin releasing hormone binding protein
18	ENSG00000160202	CRYAA	Crystallin, alpha A
19	ENSG00000172346	CSDC2	Cold shock domain containing C2, RNA binding
20	ENSG00000136943	CTSL2	Cathepsin L2
21	ENSG00000165659	DACH1	Dachshund homolog 1 (Drosophila)
22	ENSG00000181418	DDN	Dendrin
23	ENSG00000130226	DPP6	Dipeptidyl-peptidase 6
24	ENSG00000013016	EHD3	EH-domain containing 3
25	ENSG00000164035	EMCN	Endomucin
26	ENSG00000120658	ENOX1	Ecto-NOX disulfide-thiol exchanger 1
27	ENSG00000187017	ESPN	Espin
28	ENSG00000163586	FABP1	Fatty acid binding protein 1, liver
29	ENSG00000039523	FAM65A	Family with sequence similarity 65, member A
30	ENSG00000155816	FMN2	Formin 2
31	ENSG00000153303	FRMD1	FERM domain containing 1
32	ENSG00000053108	FSTL4	Follistatin-like 4

33	ENSG00000160282	FTCD	Formiminotransferase cyclodeaminase
34	ENSG00000130700	GATA5	GATA binding protein 5
35	ENSG00000121743	GJA3	Gap junction protein, alpha 3, 46kDa
36	ENSG00000116983	HPCAL4	Hippocalcin like 4
37	ENSG00000158104	HPD	4-hydroxyphenylpyruvate dioxygenase
38	ENSG00000123496	IL13RA2	Interleukin 13 receptor, alpha 2
39	ENSG00000115602	IL1RL1	Interleukin 1 receptor-like 1
40	ENSG00000027644	INSRR	Insulin receptor-related receptor
41	ENSG00000167755	KLK6	Kallikrein-related peptidase 6
42	ENSG00000169035	KLK7	Kallikrein-related peptidase 7
43	ENSG00000136944	LMX1B	LIM homeobox transcription factor 1, beta
44	ENSG00000234456	MAGI2-AS3	MAGI2 antisense RNA 3
45	ENSG00000196549	MME	Membrane metallo-endopeptidase
46	ENSG00000240666	MME-AS1	MME antisense RNA 1
47	ENSG00000205358	MT1H	Metallothionein 1H
48	ENSG00000173376	NDNF	Neuron-derived neurotrophic factor
49	ENSG00000163531	NFASC	Neurofascin
50	ENSG00000116044	NFE2L2	Nuclear factor (erythroid-derived 2)-like 2
51	ENSG00000161270	NPHS1	Nephrosis 1, congenital, Finnish type (nephrin)
52	ENSG00000116218	NPHS2	Nephrosis 2, idiopathic, steroid-resistant (podocin)
53	ENSG00000168743	NPNT	Nephronectin
54	ENSG00000162631	NTNG1	Netrin G1
55	ENSG00000138315	OIT3	Oncoprotein induced transcript 3
56	ENSG00000171759	PAH	Phenylalanine hydroxylase
57	ENSG00000067225	PKM	Pyruvate kinase, muscle
58	ENSG00000153246	PLA2R1	Phospholipase A2 receptor 1, 180kDa
59	ENSG00000128567	PODXL	Podocalyxin-like
60	ENSG00000105227	PRX	Periaxin
61	ENSG00000160801	PTH1R	Parathyroid hormone 1 receptor
62	ENSG00000153707	PTPRD	Protein tyrosine phosphatase, receptor type, D
63	ENSG00000151490	PTPRO	Protein tyrosine phosphatase, receptor type, O
64	ENSG00000164520	RAET1E	Retinoic acid early transcript 1E
65	ENSG00000143839	REN	Renin
66	ENSG00000235366	RP11-154C3.2	Uncharacterized protein

67	ENSG00000256029	RP11-190A12.7	Uncharacterized protein
68	ENSG00000258283	RP11-386G11.3	Uncharacterized protein
69	ENSG00000255509	RP11-445F12.1	Uncharacterized protein
70	ENSG00000261788	RP11-480G7.1	Uncharacterized protein
71	ENSG00000230027	RP11-550H2.2	Uncharacterized protein
72	ENSG00000255895	RP11-656E20.3	Uncharacterized protein
73	ENSG00000248115	RP11-752D24.2	Uncharacterized protein
74	ENSG00000263146	RP11-849I19.1	Uncharacterized protein
75	ENSG00000259969	RP11-999E24.3	Uncharacterized protein
76	ENSG00000243988	RPS24P17	Ribosomal protein S24 pseudogene 17
77	ENSG00000136546	SCN7A	Sodium channel, voltage-gated, type VII, alpha subunit
78	ENSG00000100665	SERPINA4	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 4
79	ENSG00000074803	SLC12A1	Solute carrier family 12 (sodium/potassium/chloride transporters), member 1
80	ENSG00000070915	SLC12A3	Solute carrier family 12 (sodium/chloride transporters), member 3
81	ENSG00000149452	SLC22A8	Solute carrier family 22 (organic anion transporter), member 8
82	ENSG00000091137	SLC26A4	Solute carrier family 26, member 4
83	ENSG00000186335	SLC36A2	Solute carrier family 36 (proton/amino acid symporter), member 2
84	ENSG00000186198	SLC51B	Solute carrier family 51, beta subunit
85	ENSG00000010379	SLC6A13	Solute carrier family 6 (neurotransmitter transporter, GABA), member 13
86	ENSG00000092068	SLC7A8	Solute carrier family 7 (amino acid transporter light chain, L system), member 8
87	ENSG00000137834	SMAD6	SMAD family member 6
88	ENSG00000167941	SOST	Sclerostin
89	ENSG00000107742	SPOCK2	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 2
90	ENSG00000184005	ST6GALNAC3	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 3
91	ENSG00000118526	TCF21	Transcription factor 21
92	ENSG00000261787	TCF24	Transcription factor 24
93	ENSG00000069702	TGFBR3	Transforming growth factor, beta receptor III
94	ENSG00000165685	TMEM52B	Transmembrane protein 52B
95	ENSG00000159173	TNNI1	Troponin I type 1 (skeletal, slow)
96	ENSG00000118194	TNNT2	Troponin T type 2 (cardiac)
97	ENSG00000134198	TSPAN2	Tetraspanin 2
98	ENSG00000092445	TYRO3	TYRO3 protein tyrosine kinase
99	ENSG00000243284	VSIG8	V-set and immunoglobulin domain containing 8

100	ENSG00000060237	WNK1	WNK lysine deficient protein kinase 1
101	ENSG00000184937	WT1	Wilms tumor 1

TRANSLATE – TRANScriptome of renaL humAn TissueE Study, TCGA – Tissue Cancer Genome Atlas

Table S8. Associations between clinic blood pressure and non-FGF1 mRNAs co-expressed with FGF1 – next-generation RNA-sequencing analysis in TRANSLATE Study.

No	Transcript name	Ensembl transcript ID	SBP		DBP	
			B-coefficient (SE)	P-value	B-coefficient (SE)	P-value
1	AC074286.1-001	ENST00000397057	0.35 (0.09)	0.0005	0.56 (0.14)	0.0003
2	AC074286.1-004	ENST00000430416	0.21 (0.13)	0.108	0.41 (0.19)	0.0422
3	AC074286.1-005	ENST00000443132	0.11 (0.04)	0.0121	0.20 (0.07)	0.0057
4	ACOX2-013	ENST00000481527	0.74 (0.26)	0.0092	1.08 (0.42)	0.0161
5	ALB-006	ENST00000484992	1.06 (0.50)	0.0448	1.80 (0.79)	0.0295
6	ALDOB-001	ENST00000374855	352.84 (122.70)	0.0074	437.69 (203.88)	0.0402
7	AXDND1-006	ENST00000489080	0.50 (0.17)	0.0065	0.76 (0.27)	0.0089
8	C14orf37-001	ENST00000267485	0.03 (0.02)	0.0498	0.04 (0.03)	0.1244
9	CALB1-001	ENST00000265431	13.85 (4.67)	0.006	22.86 (7.28)	0.0038
10	CAMK2A-001	ENST00000348628	0.01 (0.00)	0.0474	0.01 (0.01)	0.0863
11	CLIC5-003	ENST00000339561	0.52 (0.21)	0.0215	0.71 (0.35)	0.0494
12	CLIC5-201	ENST00000544153	0.02 (0.01)	0.0122	0.02 (0.01)	0.0692
13	CRB2-201	ENST00000373629	0.03 (0.01)	0.0326	0.04 (0.02)	0.0368
14	CRHBP-001	ENST00000274368	0.80 (0.23)	0.0014	0.97 (0.39)	0.0182
15	CRYAA-001	ENST00000291554	6.58 (2.53)	0.0145	10.39 (4.00)	0.0144
16	CRYAA-004	ENST00000398132	2.50 (0.77)	0.0028	4.07 (1.20)	0.0019
17	CRYAA-005	ENST00000468016	3.11 (1.01)	0.0045	4.73 (1.61)	0.0064
18	DACH1-001	ENST00000305425	0.04 (0.02)	0.0172	0.06 (0.03)	0.0551
19	DDN-001	ENST00000421952	0.22 (0.09)	0.0193	0.35 (0.14)	0.02
20	DPP6-001	ENST00000404039	0.06 (0.03)	0.0334	0.08 (0.04)	0.0434
21	EHD3-001	ENST00000322054	0.18 (0.08)	0.0228	0.24 (0.12)	0.0604
22	EHD3-201	ENST00000541626	0.11 (0.04)	0.0128	0.15 (0.07)	0.0306
23	FMN2-001	ENST00000319653	0.01 (0.00)	0.0103	0.02 (0.01)	0.0085
24	IL1RL1-002	ENST00000311734	0.13 (0.06)	0.0554	0.23 (0.10)	0.0245
25	INSRR-001	ENST00000368195	0.03 (0.01)	0.0216	0.05 (0.02)	0.0271
26	KLK6-003	ENST00000376851	0.19 (0.08)	0.0289	0.32 (0.13)	0.0184
27	KLK7-001	ENST00000595820	0.04 (0.02)	0.0226	0.06 (0.03)	0.0502
28	LMX1B-202	ENST00000425646	0.02 (0.01)	0.0175	0.03 (0.01)	0.0403
29	MAGI2-AS3-002	ENST00000429408	0.01 (0.00)	0.0332	0.01 (0.01)	0.0993
30	MAGI2-AS3-003	ENST00000414797	0.11 (0.04)	0.0046	0.17 (0.06)	0.0065

31	MAGI2-AS3-007	ENST00000452320	0.11 (0.04)	0.013	0.17 (0.07)	0.0168
32	MME-001	ENST00000460393	3.84 (1.53)	0.0175	5.66 (2.44)	0.0276
33	MME-003	ENST00000382989	0.12 (0.05)	0.0176	0.16 (0.08)	0.038
34	MME-AS1-001	ENST00000484721	1.30 (0.43)	0.0055	1.98 (0.69)	0.0074
35	NDNF-001	ENST00000379692	0.38 (0.14)	0.0092	0.64 (0.21)	0.0054
36	NFASC-205	ENST00000367169	0.05 (0.02)	0.019	0.07 (0.03)	0.0347
37	NFE2L2-015	ENST00000588123	0.19 (0.06)	0.0053	0.32 (0.10)	0.0022
38	NPHS1-001	ENST00000378910	0.54 (0.23)	0.0289	0.85 (0.37)	0.0296
39	NPHS2-001	ENST00000367615	2.66 (0.90)	0.0061	4.14 (1.42)	0.0068
40	NPNT-001	ENST00000379987	0.39 (0.17)	0.0315	0.55 (0.28)	0.0564
41	NTNG1-001	ENST00000370074	0.07 (0.02)	0.0033	0.10 (0.04)	0.0108
42	PAH-001	ENST00000553106	0.98 (0.35)	0.0084	1.52 (0.55)	0.0095
43	PAH-002	ENST00000307000	14.53 (5.10)	0.0079	22.75 (8.07)	0.0085
44	PLA2R1-001	ENST00000283243	0.06 (0.03)	0.0462	0.10 (0.05)	0.0441
45	PLA2R1-002	ENST00000392771	0.11 (0.04)	0.0145	0.15 (0.07)	0.0366
46	PODXL-201	ENST00000537928	0.60 (0.28)	0.0379	0.89 (0.44)	0.051
47	PTPRD-206	ENST00000397617	0.06 (0.02)	0.0309	0.10 (0.04)	0.0131
48	PTPRO-002	ENST00000348962	0.24 (0.08)	0.006	0.36 (0.13)	0.0102
49	RAET1E-003	ENST00000532335	0.02 (0.01)	0.0085	0.03 (0.01)	0.0163
50	RP11-154C3.2-001	ENST00000435067	0.34 (0.09)	0.0009	0.51 (0.15)	0.0017
51	RP11-550H2.2-001	ENST00000434540	0.06 (0.03)	0.0873	0.11 (0.05)	0.0421
52	RP11-656E20.3-001	ENST00000541344	0.84 (0.30)	0.0085	1.18 (0.48)	0.0203
53	RP11-752D24.2-001	ENST00000510351	0.06 (0.02)	0.0162	0.11 (0.03)	0.004
54	RP11-752D24.2-003	ENST00000504048	0.05 (0.01)	0.0008	0.08 (0.02)	0.0004
55	RP11-849I19.1-001	ENST00000575722	0.10 (0.05)	0.0468	0.18 (0.08)	0.0332
56	RP11-849I19.1-002	ENST00000572007	0.07 (0.03)	0.0224	0.12 (0.05)	0.0114
57	RPS24P17-001	ENST00000479895	0.40 (0.14)	0.006	0.69 (0.21)	0.0025
58	SCN7A-001	ENST00000409855	0.01 (0.01)	0.0824	0.02 (0.01)	0.0408
59	SLC12A1-010	ENST00000559723	0.24 (0.11)	0.03	0.32 (0.17)	0.0727
60	SLC12A3-001	ENST00000438926	2.85 (0.96)	0.0058	4.74 (1.49)	0.0035
61	SLC12A3-003	ENST00000262502	3.02 (0.99)	0.0047	4.82 (1.55)	0.0042
62	SLC12A3-007	ENST00000563352	1.05 (0.38)	0.0095	1.80 (0.58)	0.0044
63	SLC22A8-001	ENST00000336232	18.75 (7.64)	0.0203	29.80 (12.05)	0.0194
64	SLC36A2-201	ENST00000450886	2.36 (0.92)	0.0156	3.95 (1.43)	0.01

65	SLC7A8-008	ENST00000397310	3.02 (1.27)	0.0242	4.48 (2.03)	0.0349
66	SOST-001	ENST00000301691	0.33 (0.13)	0.0156	0.54 (0.20)	0.0136
67	SPOCK2-004	ENST00000373109	0.02 (0.01)	0.0182	0.02 (0.01)	0.0844
68	TCF24-001	ENST00000563496	0.01 (0.00)	0.0202	0.02 (0.01)	0.0049
69	TGFBR3-201	ENST00000212355	0.15 (0.07)	0.0396	0.21 (0.11)	0.0603
70	TMEM52B-005	ENST00000543484	0.59 (0.25)	0.0237	0.88 (0.39)	0.0338
71	TNNT2-001	ENST00000367322	0.46 (0.16)	0.0076	0.72 (0.25)	0.0082
72	TSPAN2-001	ENST00000369516	0.05 (0.02)	0.0175	0.07 (0.03)	0.0457
73	TYRO3-001	ENST00000263798	0.15 (0.05)	0.0052	0.24 (0.08)	0.0049
74	WNK1-201	ENST00000340908	0.43 (0.11)	0.0005	0.62 (0.18)	0.0018
75	WT1-004	ENST00000452863	0.04 (0.02)	0.014	0.06 (0.03)	0.0244

Data are β-coefficients, standard errors (SE) and level of statistical significance (P-value) from linear regression models wherby clinic blood pressure (adjusted for treatment) was a dependent variable, and mRNA expression level (in TPM units) for individual mRNAs, age, sex and BMI were independent parameters, TRANSLATE – TRANScriptome of renaL humAn TissueE Study

Table S9. FGF1-co-expressed genes that show association with clinic blood pressure in TRANSLATE Study and have direct prior annotation to blood pressure regulation either through Gene Ontology (GO:0008217) and its ancestor terms or by manual data mining.

No	Gene symbol	Gene name	Annotation	Relevance to blood pressure regulation	Direction of association with expression of FGF1
1	MME	Membrane metallo-endopeptidase	GO	● glycoprotein particularly abundant in kidney, where it is present on glomerular epithelium and the brush border of proximal tubules ● acts as a neutral endopeptidase cleaving peptides at the amino side of hydrophobic residues ● drives catabolism of vasoactive peptides involved in diuresis and natriuresis including natriuretic peptides, angiotensin I, bradykinin, and endothelin-1 ● pharmacological inhibition of MME was/is used in therapy of hypertension and heart failure (candoxatril, omapatrilat)	+
2	PTPRO	Protein tyrosine phosphatase, receptor type, O	GO	● encodes a protein known as Glomerular Epithelial Protein 1 ● inhibits cell proliferation and facilitates apoptosis ● possesses tyrosine phosphatase activity ● plays a role in regulating the glomerular pressure/filtration rate relationship through an effect on podocyte structure and function	+
3	REN	Renin	GO	● rate-limiting enzyme of renin-angiotensin system - responsible for conversion of angiotensinogen into angiotensin I ● an activator of the main pathway of blood pressure regulation ● in the kidney expressed primarily within the juxta-glomerular apparatus (from where it is released into circulation) and the distal nephron (local renin-angiotensin system) ● a target for pharmacological antihypertensive treatment (aliskiren)	+
4	SLC12A3	Solute carrier family 12 (sodium/chloride transporters), member 3	Manual	● encodes thiazide-sensitive sodium-chloride co-transporter (NCCT) ● key mediator of sodium and chloride reabsorption - it	+

				accounts for a significant fraction of renal sodium reabsorption ● expressed primarily within the distal portion of the tubular epithelium ● genetic mutations in this gene are known to cause Mendelian form of low blood pressure (Gitelman syndrome) ● a target for thiazide diuretics	
5	WNK1	WNK lysine deficient protein kinase 1	GO	● member of the WNK (With No K, K = lysine residue) family of serine-threonine kinases ● a key regulator of the Na-Cl co-transporter, NCCT (encoded by SLC12A3 gene) responsible for sodium reabsorption in the distal convoluted tubule and the linked process of potassium secretion by the renal outer medullary potassium channel ● mutations in this gene are recognised cause of pseudohypoaldosteronism type 2 (PHA2), also known as Gordon's syndrome (an autosomal dominant disorder of elevated blood pressure hyperkalaemia, despite normal renal glomerular filtration)	+

GO – gene ontology, TRANSLATE – TRANScriptome of renaL humAn TissueE Study, + stands for positive

Table S10. Enrichment for glomerular expression among genes associated with renal expresion of FGF1 and with the strongest relevance to blood pressure regulation.

Number	Gene	Glomerular enrichment	References
	FGF1	++++	**21-24**
1	MME	++++	21-24
2	PTPRO	++++	21-24
3	REN	+	23
4	SLC12A3		
5	WNK1		

+ refers to the positive enrichement in one in four referenced resources

Supplementary Figures

Figure S1. Regional linkage disequilibrium plot of rs152524 with 1Mb-surrounding chromosomal region. On Y axis – r^2 – a coefficient of linkage disequilibrium; on X axis – single nucleotide polymorphisms from 1000 Genomes pilot 1 project within 500 Kb distance on each side of rs152524 – information based on CEU population; in grey – single nucleotide polymorphisms in a weak linkage disequilibrium with rs152524; in yellow/orange – single nucleotide polymorphisms in a moderately strong linkage disequilibrium with rs152524; in red – single nucleotide polymorphisms in the highest linkage disequilibrium with rs152524; genes in the regions are shown as grey bars; above in red – gene symbols.

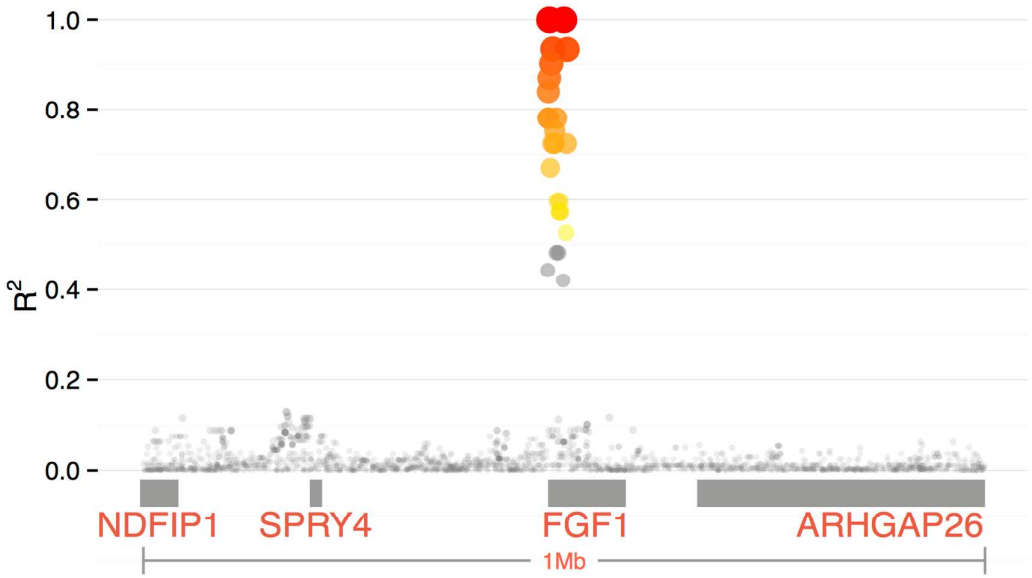


Figure S2. Partner genes of FGF1 in the kidney – next-generation RNA-sequencing in TRANScriptome of renaL humAn TissueE (TRANSLATE) Study. Outermost circle – symbols of 506 genes whose transcripts are associated with renal expression of FGF1; first circle below – level of renal expression for each partner genes (in $\log_2$ TPM+1 values) whereby white – lowest expression and navy blue – highest expression; second circle below – level of co-expression (measured as β-coefficient from linear regression) between each partner gene and FGF1 whereby dark brown – strong positive co-expression and beige – weak positive co-expression; third circle below – level of statistical significance (measured as $-\log_{10}$ P-value from linear regression) for co-expression between each partner gene and FGF1, whereby white – strong statistical significance and pink – weak statistical significance; most inner circle – level of connectivity of partner genes whereby dark red – highly connective genes, orange – genes with low connectivity.

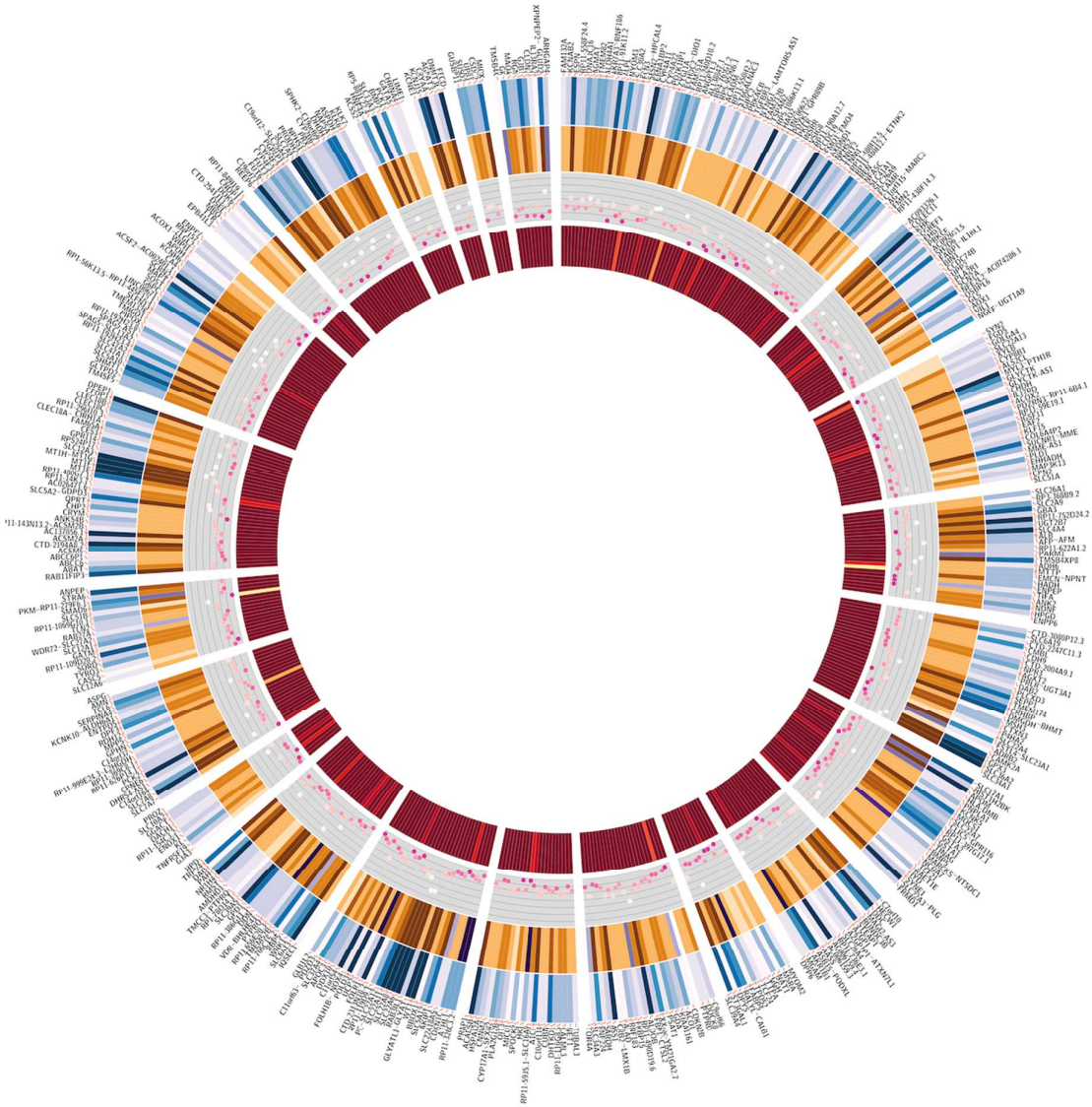


Figure S3. Replicated partner mRNAs of FGF1 in the kidney – next-generation RNA-sequencing in The Cancer Genome Atlas resource (TCGA). Replicated mRNAs – mRNAs associated with expression of FGF1 in both the TRANScriptome of renaL humAn TissueE Study and TCGA; outermost circle – symbols of 126 mRNAs ordered in circular manner; first circle below – level of renal expression for each replicated partner mRNA (in $\log_2$ TPM+1 values) whereby white – lowest expression and navy blue – highest expression; second circle below – level of co-expression (measured as β-coefficient from linear regression) between each partner mRNA and FGF1 mRNA whereby dark brown – strong positive co-expression and beige – weak positive co-expression; third circle below – level of statistical significance (measured as $-\log_{10}$ P-value from linear regression) for co-expression between each partner mRNA and FGF1 mRNA, whereby white – strong statistical significance and pink – weak statistical significance; most inner circle – level of connectivity of partner mRNAs whereby dark red – highly connective mRNAs, orange – mRNAs with low connectivity; inside – co-expression between selected mRNAs relevant to blood pressure regulation.

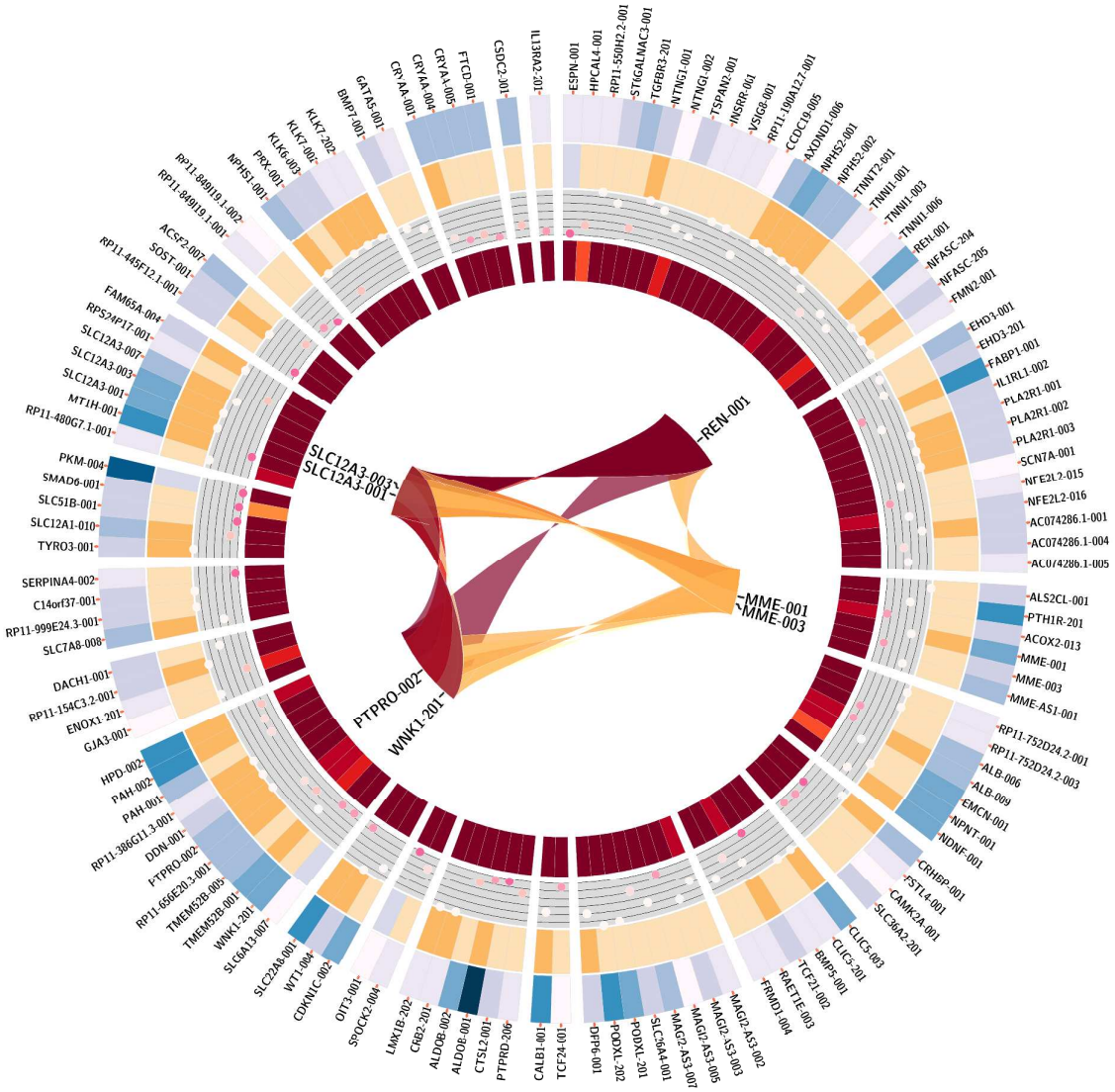
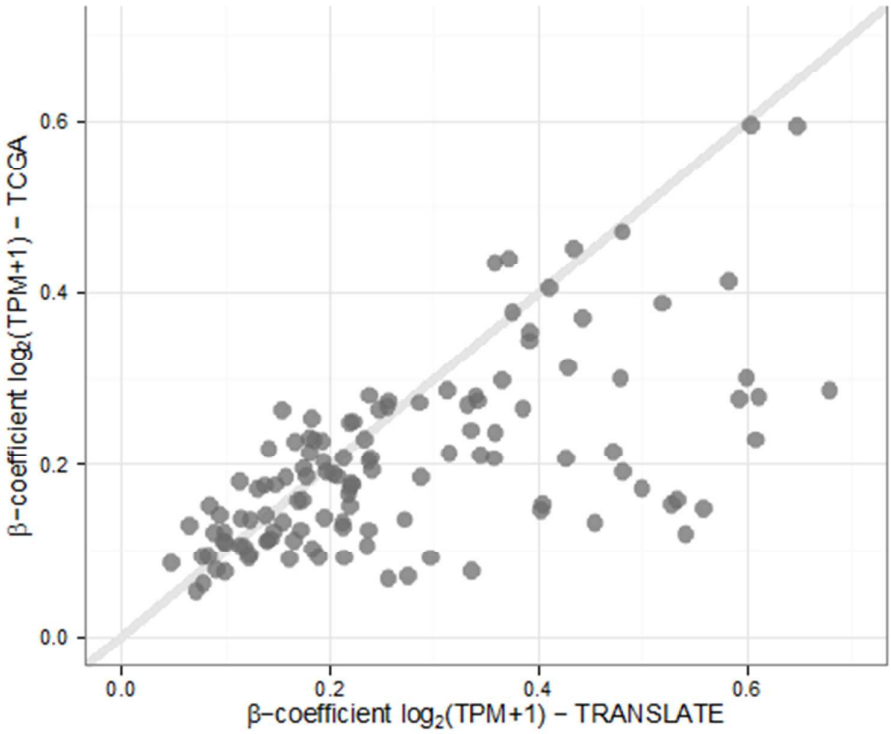


Figure S4. A total of 126 mRNAs co-expressed with FGF1 in the human kidney – consistency in the regression β-coefficient (as a measure of association of FGF1 mRNA with partner mRNAs) between the discovery population (TRANScriptome of renaL humAn TissueE – TRANSLATE Study) and the replication resource (The Cancer Genome Atlas – TCGA), $\log_2$ TPM+1 – unit of expression from next-generation RNA-sequencing.



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