RESEARCH

Fibroblast growth factor-23 rs7955866 polymorphism and risk of chronic kidney disease

Yaser Aly Ammar[1]*[ID], Dalia Aly Maharem[1], Amira Hussein Mohamed[1], Gihane Ibrahim Khalil[2], Riham Said Shams-Eldin[3] and Fatma Ibrahim Dwedar[3]

Abstract

Background: A missense gain-of-function fibroblast growth factor-23 (*FGF23*) gene single nucleotide polymorphism (SNP) (rs7955866) has been associated with FGF23 hypersecretion, phosphaturia, and bone disease. Excess circulating FGF23 was linked with atherosclerosis, hypertension, initiation, and progression of chronic kidney disease (CKD).

Methods: The study included 72 CKD stage 2/3 Egyptian patients (27–71 years old, 37 females) and 26 healthy controls matching in age and sex. Repeated measures of blood pressure were used to quantify hypertension on a semiquantitative scale (grades 0 to 5). Fasting serum urea, creatinine, uric acid, total proteins, albumin, calcium, phosphorus, vitamin D3, intact parathyroid hormone (iPTH), and intact FGF23 (iFGF23) were measured. DNA extracted from peripheral blood leucocytes was used for genotyping of *FGF23* rs7955866 SNP using the TaqMan SNP genotyping allelic discrimination method.

Results: Major causes of CKD were hypertension, diabetic kidney disease, and CKD of unknown etiology. There was no significant difference in minor allele (A) frequency between the studied groups (0.333 in GI and 0.308 in GII). Median (IQR) serum iFGF23 was significantly higher in GI [729.2 (531.9–972.3)] than in GII [126.1 (88.5–152.4)] pg/mL, $P < 0.001$. Within GI, the minor allele (A) frequency load, coded for codominant inheritance, had a significant positive correlation with both hypertension grade ($r = 0.385$, $P = 0.001$) and serum iFGF23 ($r = 0.259$, $P = 0.028$). Hypertension grade had a significant positive correlation with serum phosphorus and iFGF23.

Conclusions: For the first time in an Egyptian cohort, we report a relatively high frequency of the rs7955866 SNP. It may remain dormant or become upregulated in response to some environmental triggers, notably dietary phosphorus excess, leading to increased circulating iFGF23 with ensuing hypertension and/or renal impairment. Subjects with this SNP, particularly in the homozygous form, are at increased risk for CKD of presumably "unknown" etiology, with a tendency for early onset hypertension and increased circulating iFGF23 out of proportion with the degree of renal impairment. Large-scale population studies are needed to confirm these findings and explore the role of blockers of the renin–angiotensin–aldosterone system and sodium chloride cotransporters in mitigating hypertension associated with FGF23 excess.

Keywords: Chronic kidney disease, Fibroblast growth factor-23, Gene polymorphism, Hypertension, Phosphorus

*Correspondence: yaser_ammar88@yahoo.com
[1] Internal Medicine Department, Medical Research Institute, Alexandria University, Alexandria, Egypt
Full list of author information is available at the end of the article

Background

Chronic kidney disease (CKD), defined as kidney damage or glomerular filtration rate (GFR) < 60 mL/min/1.73 m^2 for ≥ 3 months [1], is a major clinical and public health problem, afflicting about one-tenth of the population

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worldwide [2]. It is a complex, inherently progressive condition that drastically reduces a person's productivity, impairs quality of life, and increases rates of hospitalizations and mortality, particularly in low- and middle-income countries [3]. Parallel with renal dysfunction, these patients typically develop CKD-mineral bone disorder (CKD-MBD); which is a complex syndrome encompassing abnormal bone histology, perturbed calcium and phosphorus metabolism, and progressive extraskeletal (predominantly vascular) calcification (VC) and accelerated atherosclerotic cardiovascular disease [4]. In a significant proportion of patients, CKD cannot be attributed to an identifiable cause, the so-called CKD of unknown etiology (UKN). In these cases, trying to identify the as yet unknown, possibly genetic, underlying factors is tempting and might open avenues for earlier recognition and more specific management [5].
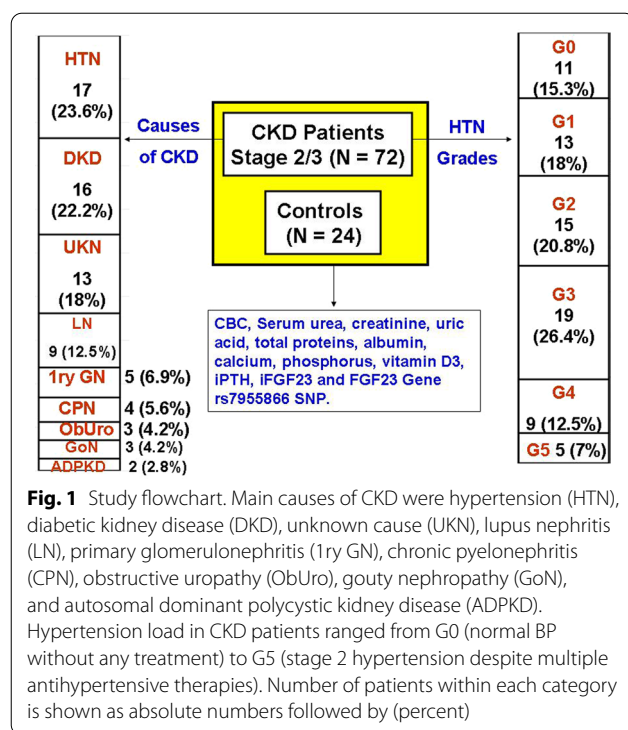
Fibroblast growth factor 23 (FGF23), the master regulator of phosphorus homeostasis, is a 251 amino acid polypeptide, primarily secreted by osteocytes and osteoblasts in response to phosphorus loading. In the renal tubules, it forms a trimeric signaling complex with FGF23 receptor (FGFR) and α-klotho coreceptor [6] that efficiently promotes phosphaturia, both directly (by suppressing apical epithelial expression of type 2 sodium phosphate cotransporters responsible for proximal tubular phosphate reabsorption), and indirectly (by inhibiting one α-hydroxylase activation of vitamin D and promoting its inactivation by 24-hydroxylase) [7, 8]. Cleavage of the intact FGF23 (iFGF23) between Arg^{179} and Ser^{180} generates two (n- and c-terminal) inactive fragments. Excess cFGF23 competitively inhibits the biologically active, full-length iFGF23. Therefore, cleavage of iFGF23 is an important post-translational regulatory event [9]. Missense gain-of-function mutations affecting the cleavage site might result in iFGF23 molecules becoming more resistant to proteolytic cleavage, increasing their circulating level [10, 11]. In fact, the *FGF23* gene was first identified by positional cloning on chromosome 12p13.3 as being responsible for autosomal dominant hypophosphatemic rickets (ADHR), a rare hereditary disorder, characterized by increased circulating FGF23, hypophosphatemia, hypovitaminosis D, and impaired skeletal development [12].

By direct sequencing of the three coding exons and the two flanking introns, screening for *FGF23* gene variation in 183 Finnish children and adolescents identified nine variants. A relatively common exon 3 variant (rs7955866) occurred in a heterozygote form in 37 (one-fifth) of these subjects [13]. In this variant, the normal ACG triplet (encoding threonine) at codon 239 changes to an ATG triplet (encoding methionine), with a corresponding change from guanine to adenine on the opposite DNA strand [14]. This variant was designated c.716C > T, g.4370383G > A, or p.T239M [15]. In vitro, FGF23 levels were significantly higher in conditioned media containing human embryonic kidney (HEK293) cells transfected with *FGF23*-239M plasmids, compared with media of cells expressing the wild FGF23-239T protein [16]. However, in instrumental analysis, *FGF23* gene variance was a weak determinant of circulating FGF23 and phosphate concentrations [13]. ADHR mutations have variable penetrance and age at clinical presentation, with spontaneous resolution of the renal phosphate wasting in some cases [17]. Among 42 subjects with ADHR mutations, only 24% and 9% had increased c-terminal and iFGF23 levels, respectively. The iFGF23 increments and ADHR features tended to wax and wane over time [18].

FGF23 is a pleotropic hormone with a host of klotho-dependent and klotho-independent functions [19, 20]. Early in CKD, an adaptive increase of circulating FGF23 precedes alterations of other CKD-MBD markers and guards against hyperphosphatemia [21]. With CKD progression, serum iFGF23 continues to increase due to progressive hyperphosphatemia [22], hyperparathyroidism [23], klotho deficiency [24], as well as decreased renal clearance [25]. The highest ever reported serum iFGF23 values (> 10,000 pg/mL) occur in patients with advanced CKD and constitute a significant harbinger of end-stage renal disease (ESRD) and mortality [26, 27]. Several recent reports have linked circulating FGF23 excess with an expanding spectrum of disease conditions including CKD initiation [28] and progression [29, 30], renal allograft loss [31, 32], chronic inflammation [33], immune deficiency [34], insulin resistance [35], dyslipidemia [36], early [37, 38] and advanced atherosclerosis [39, 40], VC [41], hypertension [42–44], left ventricular hypertrophy [45], heart failure [46], as well as cardiovascular and all-cause mortality [47, 48], particularly in CKD [49] and ESRD [50–53] patients. The adverse consequences of circulating FGF23 excess remained significant after adjustment for serum calcium, phosphorus, parathyroid hormone (PTH) [29], and vitamin D levels [52].

In Egypt, the prevalence of ESRD has been steadily increasing. Although this trend may reflect increased surveillance, more availability of dialysis centers, and increased longevity on treatment, a true surge of incident cases is evident and compatible with the increased prevalence of non-communicable diseases closely linked with CKD development and progression, particularly hypertension and diabetes mellitus [54]. Hypertension is highly prevalent in Egypt, with apparently suboptimal management [55–57]. It has been repeatedly recognized as the major cause of ESRD [58–60]. About one-fifth of ESRD cases are not attributable to a known factor (UKN) and may represent the tip of a large iceberg of poorly

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Fig. 1 Study flowchart. Main causes of CKD were hypertension (HTN), diabetic kidney disease (DKD), unknown cause (UKN), lupus nephritis (LN), primary glomerulonephritis (1ry GN), chronic pyelonephritis (CPN), obstructive uropathy (ObUro), gouty nephropathy (GoN), and autosomal dominant polycystic kidney disease (ADPKD). Hypertension load in CKD patients ranged from G0 (normal BP without any treatment) to G5 (stage 2 hypertension despite multiple antihypertensive therapies). Number of patients within each category is shown as absolute numbers followed by (percent)

identified CKD cases [61]. There is now ample evidence for the role of circulating FGF23 excess in occurrence and progression of hypertension [42–44] and CKD [28–30] that may be more prominent in genetically predisposed

individuals [10, 11]. Therefore, we hypothesized that gain-of-function variants of *FGF23* gene might represent unforeseen contributors to the recognizable burden of hypertensive CKD in Egypt. Accordingly, we studied the occurrence and correlates of the rs7955866 *FGF23* gene single nucleotide polymorphism (SNP) among Egyptian subjects with or without CKD.

Methods

Study design and participants

This was a cross-sectional, case–control study that involved two comparative groups (Fig. 1, Table 1):

Group 1 (G I): 72 CKD patients (27–71 years old, 37 females).

- Inclusion Criteria:

  - Adult age.
  - Having CKD stage 2 or 3, as defined by an estimated glomerular filtration rate (eGFR) of 30–89 mL/min/1.73 m^2 as per K/DOQI guidelines[62].
  - Providing a written informed consent to participate.

- Exclusion criteria: secondary hypertension, (such as patients with endocrinopathies and renovascular hypertension), urologic abnormalities potentially

Table 1 Characteristics of the study subjects

Parameter	CKD patients (N=72)	Controls (N=26)	Statistical test	P value
Male	35 (48.6%)	14 (53.8%)	Chi-square	0.647
Female	37 (51.4%)	12 (46.2%)		
Age (Y)	48 (38.8–59.3)	43 (36.5–52)	MWU	0.179
S. Creatinine (mg/dL)	1.5 (1.2–1.9)	0.9 (0.7–1)	MWU	<0.001**
GFR-EPI (mL/min/1.73)	44 (35.3–61.5)	95.5 (81.3–112)	MWU	<0.001**
Proteinuria (gm/day)	2.1 (1.2–4)	0.1 (0.1–0.1)	MWU	<0.001**
S. Urea (mg/dL)	81.44±20.6	27.77±5.9	TT	<0.001**
S. Uric Acid (mg/dL)	7.39±2.4	5.08±1.2	TT	<0.001**
S. Proteins (gm/dL)	6.4 (5.7–6.9)	6.9 (6.4–7.1)	MWU	<0.001**
S. Albumin (gm/dL)	3.2 (2.6–3.5)	3.9 (3.7–4.1)	MWU	<0.001**
S. Calcium (mg/dL)	9.09±0.9	9.35±0.6	TT	0.105
S. Phosphorus (mg/dL)	4.88±1	3.5±0.3	TT	<0.001**
S. iPTH (pg/mL)	160.5 (100–202.5)	41.5 (30–48)	MWU	<0.001**
S. Vitamin D3 (ng/mL)	14.8 (12–22)	35.2 (29.3–38.2)	MWU	<0.001**
S. iFGF23 (pg/mL)	729.2 (531.9–972.3)	126.1 (88.5–152.4)	MWU	<0.001**
Hemoglobin (gm/dL)	10.2 (9.4–11.3)	13.1 (12.9–14.3)	MWU	<0.001**
WBCs (k/uL)	7.5 (5.9–9.3)	5.9 (4–7.1)	MWU	<0.001**
Platelets (k/uL)	296.63±74.7	320.81±39.8	TT	0.043*

CKD, chronic kidney disease; S., serum; GFR-EPI, estimated glomerular filtration rate by CKD epidemiology collaboration equation; iPTH, intact parathyroid hormone; iFGF23, intact fibroblast growth factor 23; WBCs, white blood cells; k, one thousand; MWU, Mann–Whitney U test; TT, independent sample *t*-test

Data are expressed as either mean±SD or median (interquartile range), *: significant (*P* < 0.05), **: highly significant (*P* < 0.01)

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impacting urinary protein excretion or renal functions (such as vesicoureteric reflux), malignancies, and paraproteinemias.

Group 2 (G II): 26 healthy control subjects matching in age and sex.

The main causes of CKD were identified by thorough history, clinical examination, and medical record review. Blood pressure (BP) was measured by a standard technique in the upper arm while sitting comfortably. Three measures were made, one minute apart; and the average of the last two was recorded. Measurements were repeated at 2–3 visits, 2–3 weeks apart. Further office and/or home measurements were made, if required. The BP was ultimately given a score of either 0 (normal), 1 (elevated), 2 (stage 1 HTN), or 3 (stage 2 HTN), according to the 2017 ACC/AHA guidelines [63]. To account for the effect of therapy, one extra point was added if the patient achieved this level of BP with interrupted or single-agent therapy; and two points were added for patients on regular treatment with ≥ 2 agents. Patients with inconsistent findings were checked at more occasions until they were ultimately placed on a semiquantitative scale to express hypertension burden extending from 0 (normal without treatment) to 5 (stage 2 hypertension despite regular multiple antihypertensive medications).

Laboratory studies [64]
After an overnight fast, blood samples were drawn into EDTA tubes (for complete blood count and DNA extraction) and serum separator tubes that were immediately transported and centrifuged. The separated serum was kept at − 80 °C until batch analysis was made for urea, creatinine, uric acid, total proteins, albumin, calcium, phosphorus, intact parathyroid hormone (iPTH) (third-generation assay), vitamin D3, and intact FGF23 (iFGF23). The latter was tested by Kainos ELISA kit that targets the iFGF23 molecule by utilizing two monoclonal antibodies which simultaneously capture two epitopes flanking the cleavage site of the c-terminal fragment (Kainos Laboratories, Tokyo, Japan) [65]. Estimated GFR was calculated by the CKD epidemiology collaboration (CKD-EPI) equation [66]. A 24-h urine collection was used to assess total daily urinary protein excretion.

Study of FGF23 Gene rs7955866 SNP
DNA extraction
DNA was extracted from whole blood EDTA samples using a spin column protocol [QIAamp DNA Blood Mini Kit] provided by QIAGEN Inc. (Qiagen, Hilden, Germany) according to manufacturer's instructions. Extracted DNA was kept at − 80 °C till analysis.

Determination of the DNA concentration and quality
NanoDrop 2000/2000c (Thermoscientific, USA) was used to check DNA quality and quantity. Samples having poor DNA-purity or extensively fragmented DNA were excluded from the analysis [67].

Genotyping
This was performed using $40 \times$ TaqMan® predesigned SNP genotyping assay provided by Thermo Fisher Scientific, Waltham, Massachusetts, USA (Assay ID: C_25605491_10). The context sequence of the rs7955866 *FGF23* SNP was:

AGCCTTCCGGGCCC[G/A]TTCCCCCAGCGT GTTCACT.

The A allele was detected with VIC® dye and the G allele with FAM® dye.

The reaction mix was composed of 40X TaqMan® genotyping assay, TaqMan® universal PCR master mix, and nuclease-free water. The 40X predesigned SNP assay was diluted to a 20X working solution with nuclease-free water. The recommended final reaction volume per well was 20 µL for a 48-well plate (17 µL reaction mix + 3 µL DNA sample). For reaction mix preparation, 10µL of 2X TaqMan® Genotyping Master Mix, 1 µL of 20X Assay Working Solution (0.5 µL 40X TaqMan assay + 0.5 µL Nuclease free water), and 6 µL of nuclease-free water were added in each well. The total reaction volume uses 20 ng of genomic DNA. Real-time PCR was performed using Applied Biosystems StepOne™ Real-Time PCR System.

In the real-time PCR system software, an experiment or plate document was using the following thermal cycling conditions; first AmpliTaq Gold® Enzyme Activation step at 95 °C for 10 min then 40 cycles; each consisted of 15 s at 95 °C for denaturation and 1 min at 60 °C for annealing/extension. No template controls (NTC) were performed by adding 3 µl of DNase-free water into each well instead of DNA.

Post-PCR plate read and analysis
Life Technologies real-time instrument software plotted the results of the allelic discrimination data as a scatter plot of allele 1 (VIC® dye) versus allele 2 (FAM™ dye). Each well of the 48-well reaction plate was represented as an individual point on the plot. Applied Biosystems Step One™ Software was the software application used to analyze raw data from genotyping experiments created on a Life Technologies real-time PCR system.

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Statistical methods

Data were analyzed using SPSS software package version 20 (SPSS Inc., Chicago, Illinois, USA). Categorical data were expressed as absolute numbers (percentages) and compared by Chi-square or Fisher exact test. Continuous data were tested for normality using Shapiro–Wilk test. Parametric data were presented as mean ± SD and compared by independent t-test or analysis of variance (ANOVA). Nonparametric data were presented as median (interquartile range) and compared by Mann–Whitney U or Kruskal–Wallis H test. Correlations were tested by the Spearman's rank correlation coefficient. Significance was judged at the 5% level.

Fig. 2 Distribution of the 3 genotypes of FGF23 gene rs7955866 SNP in the two study groups

Results

Among CKD patients, 15.3% had normal BP without any treatment (G0), whereas 18%, 20.8%, 26.4%, 12.5%, and 7% had hypertension grades 1 through 5, respectively (Fig. 1). Compared with controls, CKD patients had significantly higher S. creatinine, urea, uric acid, phosphorus, iPTH, iFGF23, WBCs, and proteinuria, and significantly lower eGFR, serum total proteins, albumin, vitamin D3, hemoglobin, and platelets (Table 1).

There was no significant difference in the minor allele frequency (MAF) of the rs7955866 *FGF23* SNP (A allele) between CKD patients (0.333) and controls (0.308) with any mode of inheritance (all $P > 0.7$). (Table 2, Fig. 2).

CKD patients with the homozygote variant "AA" were compared with the other two genotypes, either separately (Table 3, Figs. 3, 4), or considering these two genotypes as one group "GG/GA" (not shown). In both instances, "AA" CKD patients had significantly higher hypertension grade, significantly higher serum iFGF23, and significantly lower serum albumin. The "A" allele load had a significant positive correlation with hypertension grade, which was consistent across the 3 inheritance modes (Table 4). Coded for some inheritance modes only, the "A" allele load had a significant but weak positive correlation with serum iFGF23, and a significant b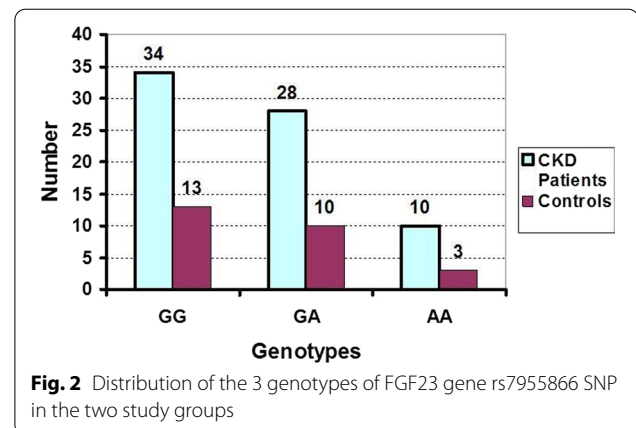ut weak negative correlation with serum albumin and WBCs. In the codominant and recessive modes, correlations of the "A" allele load with serum iFGF23 persisted, after controlling for GFR, serum phosphorus, and iPTH. Hypertension grade had a significant but weak positive correlation with serum phosphorus and iFGF23 (Table 5). Having "AA" genotype modestly increased the risk for having hypertension (OR: 1.73, CI 0.2–15.21, $P = 0.621$) and for being in the highest serum iFGF23 tertile (OR: 2.26, CI 0.59–8.75, $P = 0.236$).

Discussion

Genetic variants affecting circulating FGF23 level may occur within the *FGF23* gene itself or within loci controlling vitamin D and phosphate metabolism [68]. Most previous studies of the rs7955866 SNP (c.716C > T, g.4370383G > A, p.T239M) have reported a MAF < 0.2 [14, 69, 70]. For the first time in an Egyptian cohort, we report a remarkably higher MAF (0.327), which was insignificantly higher among CKD subjects compared with controls (0.333 versus 0.308, respectively). Both experimental [16] and clinical [14, 71] studies have established that the missense, gain-of-function ADHR mutations may result in increased tissue or circulating FGF23 levels that are attributed to the resistance of the variant molecule to proteolytic cleavage [10, 11]. However,

Table 2 Comparison between CKD patients and controls regarding FGF23 RS7955866 polymorphism genotypes

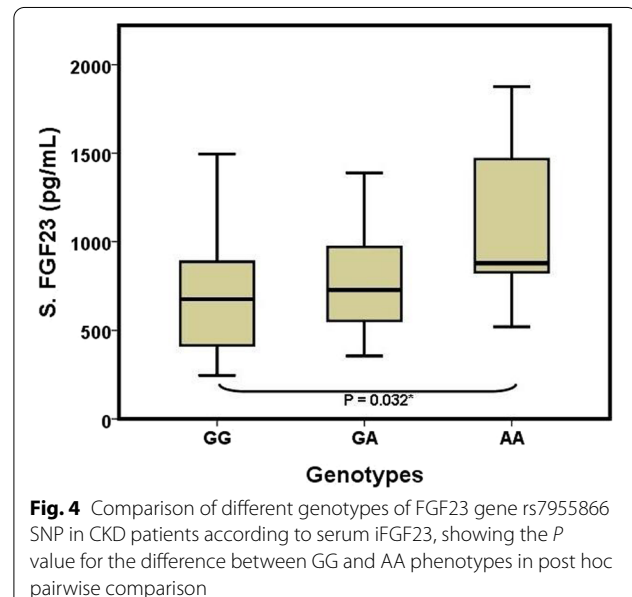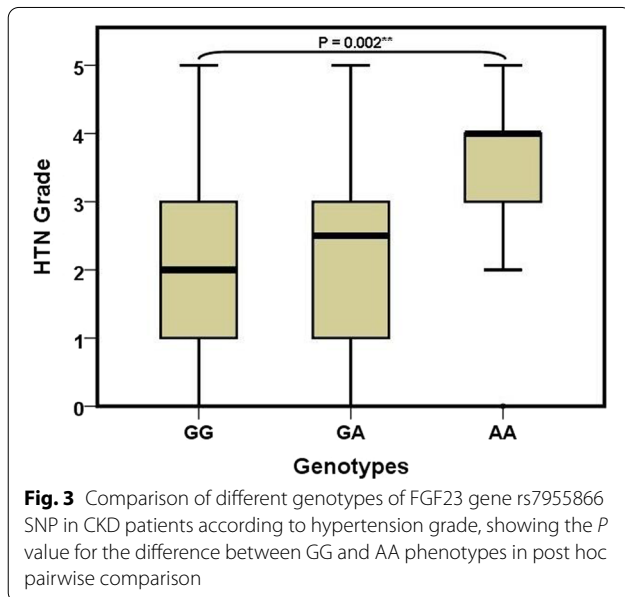| Inheritance mode	Genotypes	CKD patients (*N* = 72)	Controls (*N* = 26)	Total (*N* = 98)	*P* value
Codominance	GG	34 (47.2%)	13 (50%)	47	0.946
	GA	28 (38.9%)	10 (38.5%)	38	
	AA	10 (13.9%)	3 (11.5%)	13	
G Dominance	GG–GA	62 (86.1%)	23 (88.5%)	86	0.762
	AA	10 (13.9%)	3 (11.5%)	13	
A Dominance	GG	34 (47.2%)	13 (50%)	47	0.808
	GA–AA	38 (52.8%)	13 (50%)	51	

CKD, chronic kidney disease; FGF23, fibroblast growth factor 23

Table 3 Comparison between CKD patients regarding FGF23 RS7955866 polymorphism genotypes

Parameter	GG (N = 34)	GA (N = 28)	AA (N = 10)	Statistical test	P
Hypertension Grade	2 (0.75–3)	2.5 (1–3)	4 (2.75–4.25)	KWH	0.003**
S. Creatinine (mg/dL)	1.45 (1.2–1.7)	1.65 (1.1–2)	1.50 (1.1–1.83)	KWH	0.619
GFR-EPI (mL/min/1.73)	45.68 (36.75–60.52)	42.53 (34.01–64.44)	41.84 (37.55–64.76)	KWH	0.913
Proteinuria (gm/day)	2.16 (1.17–3.96)	2.18 (1.09–4.14)	1.83 (1.19–3.96)	KWH	0.988
S. Urea (mg/dL)	79.91 ± 20.56	84.07 ± 21.83	79.30 ± 18.34	ANOVA	0.693
S. Uric Acid (mg/dL)	7.74 ± 2.43	7.33 ± 2.33	6.35 ± 2.61	ANOVA	0.278
S. Proteins (gm/dL)	6.41 ± 0.78	6.39 ± 0.79	5.95 ± 0.77	ANOVA	0.242
S. Albumin (gm/dL)	3.35 (2.58–3.73)	3.15 (2.9–3.4)	2.5 (2.45–3.05)	KWH	0.007**
S. Calcium (mg/dL)	9.04 ± 0.86	9.24 ± 0.86	8.81 ± 0.95	ANOVA	0.37
S. Phosphorus (mg/dL)	4.75 (4.10–5.93)	4.55 (3.95–5.1)	5.3 (4.13–5.78)	KWH	0.368
S. iPTH (pg/mL)	127.5 (101.3–192.3)	178 (111–221)	133.5 (74.5–237)	KWH	0.387
S. Vitamin D3 (ng/mL)	14.75 (12–19.75)	13.25 (11.13–22)	17 (14.25–26.1)	KWH	0.268
S. iFGF23 (pg/mL)	676.71 (409.49–916.85)	729.19 (544.03–970.98)	879.53 (791.83–1486.7)	KWH	0.038*
Hemoglobin (gm/dL)	10.49 ± 1.28	10.22 ± 1.20	10.42 ± 1.33	ANOVA	0.708
WBCs (k/uL)	7.75 (5.78–9.85)	7.65 (6.75–9.45)	5.78 (5.13–7.33)	KWH	0.09
Platelets (k/uL)	304.24 ± 77.12	282.18 ± 68.27	311.20 ± 84.47	ANOVA	0.416

CKD, chronic kidney disease; Hypertension grades are numbered 0 (none) through 5 (most severe); S., serum; GFR-EPI, glomerular filtration rate by CKD epidemiology collaboration equation; iPTH, intact parathyroid hormone; iFGF23, intact fibroblast growth factor 23; WBCs, white blood cells; k, one thousand; ANOVA, analysis of variance; KWH, Kruskal–Wallis H Test

Data are expressed as either mean ± SD or median (interquartile range), *: significant ($P < 0.05$), **: highly significant ($P < 0.01$)

Fig. 3 Comparison of different genotypes of FGF23 gene rs7955866 SNP in CKD patients according to hypertension grade, showing the *P* value for the difference between GG and AA phenotypes in post hoc pairwise comparison

Fig. 4 Comparison of different genotypes of FGF23 gene rs7955866 SNP in CKD patients according to serum iFGF23, showing the *P* value for the difference between GG and AA phenotypes in post hoc pairwise comparison

complex translational and post-translational FGF23 regulatory mechanisms tend to maintain its circulating levels within normal, unless the expression of the variant *FGF23* gene is stimulated by some acquired triggering factors, notably anemia, iron deficiency or hyperphosphatemia, which typically accompany renal function impairment [18, 72–74]. The differential expression of the variant *FGF23* gene was evident in the present study.

Within controls, serum iFGF23 showed neither a significant difference between different genotypes (Additional file 1: Supplemental Sheet 1), nor a significant correlation with the "A" allele load, employing different inheritance modes (Additional file 1: Supplemental Sheet 2). On the other hand, CKD patients, with their significantly lower eGFR, hemoglobin, and significantly higher serum phosphorus, displayed a significant elevation of serum iFGF23

Table 4 Statistical correlations of "A" allele load of FGF23 RS7955866 polymorphism in CKD patients

Parameter	Assumed mode of "A' allele inheritance					
	Codominant		Dominant		Recessive	
	r	P	r	P	r	P
Hypertension grade	0.385	0.001**	0.324	0.006**	0.349	0.003**
S. Creatinine	0.061	0.612	0.089	0.455	− 0.035	0.771
GFR-EPI	− 0.025	0.832	− 0.038	0.750	0.016	0.891
Proteinuria	0.018	0.881	0.019	0.876	0.008	0.949
S. Urea	0.023	0.846	0.044	0.712	− 0.037	0.759
S. Uric Acid	− 0.170	0.155	− 0.137	0.25	− 0.166	0.163
S. Proteins	− 0.124	0.3	− 0.075	0.531	− 0.181	0.128
S. Albumin	− 0.29	0.014*	− 0.199	0.094	− 0.368	0.001**
S. Calcium	0.017	0.887	0.069	0.564	− 0.113	0.344
S. Phosphorus	− 0.100	0.402	− 0.138	0.247	0.036	0.765
S. iPTH	0.015	0.901	0.066	0.58	− 0.113	0.344
S. Vitamin D3	0.094	0.431	0.036	0.767	0.189	0.113
Hemoglobin	− 0.071	0.551	− 0.092	0.44	0.012	0.923
WBCs	− 0.11	357	− 029	81	− 0.25	0.034*
Platelets	− 0.061	0.609	− 0.1	0.404	0.058	0.629
S. iFGF23	0.259	0.028*	0.194	0.102	0.29	0.014*
S. iFGF23, controlled for GFR	0.289	0.015*			0.341	0.004**
S. iFGF23, controlled for S. Phosphorus	0.311	0.008**			0.344	0.003**
S. iFGF23, controlled for S. iPTH	0.262	0.027*			0.32	0.006**

CKD, chronic kidney disease; S., serum; GFR-EPI, glomerular filtration rate by CKD epidemiology collaboration equation; iPTH, intact parathyroid hormone; iFGF23, intact fibroblast growth factor 23; WBCs, white blood cells; *r*, Spearman correlation coefficient

*: significant ($P < 0.05$), **: highly significant ($P < 0.01$)

Table 5 Other correlations of hypertension grade in CKD patients

S. Creatinine	r	0.147	S. Calcium	r	− 0.205
	P	0.217		P	0.085
GFR-EPI	r	− 0.128	S. Phosphorus	r	0.269
	P	0.282		P	0.023*
Proteinuria	r	− 0.034	S. Vitamin D3	r	− 0.195
	P	0.776		P	0.1
S. Urea	r	0.068	S. iPTH	r	0.014
	P	0.572		P	0.907
S. Uric Acid	r	− 0.2	S. iFGF23	r	0.401
	P	0.092		P	< 0.001**

CKD, chronic kidney disease; S., serum; GFR-EPI, glomerular filtration rate by CKD epidemiology collaboration equation; iPTH, intact parathyroid hormone; iFGF23, intact fibroblast growth factor 23; *r*, Spearman correlation coefficient

*: significant ($P < 0.05$), **: highly significant ($P < 0.01$)

in "AA" compared with "GG" patients. Moreover, the "A" allele load, coded for codominant and recessive modes, had significant positive correlations with serum iFGF23, which persisted after controlling for serum phosphorus, iPTH and eGFR (Table 4). The rs7955866 *FGF23* SNP may therefore be relatively dormant in healthy subjects.

Once subjects with this variant develop impaired kidney function, the variant becomes actively expressed, leading to increased circulating FGF23 with its potential multi-system morbid sequelae, including further progression of CKD, thus creating a vicious cycle. Indeed, CKD is the protype and most common disorder of secondary increased circulating iFGF23 [72]. These patients typically have a constellation of the key FGF23 secretion triggers (hyperphosphatemia, hyperparathyroidism, and klotho deficiency), in addition to the frequent occurrence of hypoxia, anemia, iron deficiency, and chronic inflammation, which are all known to upregulate *FGF23* gene expression [75, 76].

The global burden of hypertension is increasing [77], with comparatively higher prevalence but lower recognition and control rates in lower- and middle-income countries [78]. In the present study, hypertension was the most common cause of CKD, accounting for 23.6% of cases, which was matching with the role of hypertension as the leading cause of ESRD in Egypt [58–60]. Hypertension is a salient feature of CKD of whatever etiology; both conditions have a well-recognized bidirectional relation in which the kidney behaves as both a culprit and a victim [79]. Some degree of hypertension

occurred in 84.7% of CKD patients in the present study, emphasizing the importance of hypertension control as a feasible approach to delay CKD progression [80, 81]. Hypertension is a highly heritable trait, with genetic factors significantly determining the rates of its prevalence and response to therapy among different ancestral groups [82]. In the present study, "AA" CKD patients had significantly higher hypertension grade and significantly higher serum iFGF23, compared with the other genotypes. Hypertension grade had a significant positive correlation with both the "A" allele load (coded for different inheritance modes) and serum iFGF23. Therefore, harboring the "A" genotype, in a homo- or a heterozygote state, stood out as a significant risk factor for hypertension, which is mediated through circulating iFGF23 excess.

The association between increased circulating iFGF23 and hypertension has been described both in community dwellers [42, 43] and CKD patients [44]. Urinary FGF23/creatinine ratio was found to be significantly higher in 42 hypertensive children and adolescents, compared with controls; and it had a significant direct correlation with systolic BP in all study participants [83]. Circulating iFGF23 excess may be related to hypertension through its stimulant effect on the renin–angiotensin–aldosterone system (RAAS) [84]. RAAS activation may be mediated by FGF23 either by suppressing the angiotensin converting enzyme-2 (ACE2) expression in the kidney, independent of other CKD-MBD abnormalities [85], or by inducing active vitamin D (calcitriol) deficiency. FGF23 is a strong independent predictor of low calcitriol levels, even after adjustment for renal function, serum phosphorus, and calcidiol levels [86]. Calcitriol behaves as a negative RAAS regulator [87, 88], probably by reducing renin gene expression [89]. More recently, further pathogenetic associations between FGF23 and hypertension were established when its ability to augment distal renal tubular sodium reabsorption was disclosed [90]. Mice treated with an intraperitoneal injection of recombinant FGF23 developed a klotho-dependent 40% upregulation of the distal tubular sodium chloride cotransporter (NCC), reduced urinary sodium and water excretion coupled with circulatory sodium and water retention, and ensuing hypertension. Therefore, FGF23 was assigned a significant role as a sodium-retaining hormone involved in volume and BP regulation [91]. Further studies should explore whether RAAS blockers (as ACE inhibitors) and NCC blockers (as thiazide diuretics) are particularly effective in treating hypertension in the context of FGF23 excess. The hypertensive effects of iFGF23 may also stem from induction of endothelial dysfunction [37, 38] and VC [41].

The relationship between circulating iFGF23 levels and serum phosphorus is largely influenced by renal function [92]. The markedly increased circulating iFGF23 levels in patients with hereditary hypophosphatemic disorders and normal renal function are closely correlated with increased urinary fractional excretion of phosphate and hypophosphatemia [93–95]. A similar situation occurs in the early few months following renal transplantation, when increased serum iFGF23 is the primary factor responsible for post-transplant hypophosphatemia [96–98]. In patients with less striking iFGF23 elevations and normal to moderately decreased renal function (like the current study cohort, Additional file 1: Supplemental Sheet 3), serum iFGF23 has no significant correlation with serum phosphorus [99, 100]. However, as CKD progresses to ESRD, serum iFGF23 increases in a strong direct correlation with serum phosphorus [52, 53, 101]; hyperphosphatemia may then partly explain the association of iFGF23 with adverse cardiovascular outcomes [102–104]; and control of hyperphosphatemia may be a feasible approach to mitigate FGF23 excess and its morbid sequelae [105].

Hyperphosphatemia is a common finding and a major risk factor for cardiovascular events and mortality in CKD patients [106–108]. A graded association between serum phosphorus and cardiovascular disease extends to the normal serum phosphorus range and to people with normal renal function [109–111]. In the present study, serum phosphorus had a significant positive correlation with hypertension grade in the CKD patients. Previously, hyperphosphatemia has been strongly and independently associated with hypertension in hemodialysis patients [112], and with increased BP variability in patients with earlier stages of CKD [113]. It was also associated with poor response to antihypertensive therapy, irrespective of renal function status [114]. Several mechanisms explain the association between hyperphosphatemia and hypertension. Phosphorus exposure decreased endothelium-dependent vasodilatation of the brachial artery in healthy men; a similar in vitro effect was documented in rat aortic rings [115]. Similar results were recently reproduced by a study involving two counterparts of humans and rat mesenteric vessels [116]. Hyperphosphatemia may also increase endothelial production of the vasoconstrictor endotelin-1 [117], increase renin expression [118], and activate the sympathetic nervous system [119], besides playing a key role in CKD progression [120], VC and arterial stiffness [121–123]. High phosphorus diet may also stimulate FGF23 secretion, leading to RAAS activation, sodium and water retention, and consequently hypertension, in subjects with or without CKD [90]. Therefore, phosphorus overconsumption may be a dietary factor augmenting the pressor effect of the rs7955866 SNP, particularly in less-privileged communities, habitually consuming relatively low-cost processed foods having

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high inorganic phosphate content, that is almost totally absorbed [124–127].

We report also an association between the "A" allele load and lower serum albumin in the studied CKD patients (Tables 3, 4). This effect could not be explained by variations in total proteinuria, which had no relation with the occurrence of the SNP or the serum iFGF23 level. Circulating FGF23 has been correlated with the extent of proteinuria in CKD patients in some [128, 129], but not all studies [130]. It is possible that patients with the *FGF23* SNP had lower serum albumin due to higher albuminuria. Increased circulating FGF23 increases the risk of albuminuria in patients with normal kidney function to moderate CKD [30, 131]. However, urinary albumin excretion rate was not specifically assayed in the present study.

Previous studies have described an association between circulating FGF23 excess and both CKD initiation [28] and progression [29, 30]. Increased circulating iFGF23 may directly affect the glomerular endothelial function or glomerular hemodynamics, through klotho-independent signaling [38, 131]. Alternatively, FGF23 may exert its detrimental effects on renal function through its association with hypertension, atherosclerosis, and VC. The present study did not reveal a clear association between the rs7955866 SNP and all-cause CKD, although, more specifically, this SNP seemed to increase the risk of hypertension and hypertensive CKD, an association mediated by circulating FGF23 excess. In 18% of the patients, CKD could not be attributed to a definitive cause (UKN). When CKD develops in relation to a gain-of-function *FGF23* gene mutation, it is conceivable that the insidiously occurring hypertension and renal impairment would be largely asymptomatic and difficult to recognize, at least initially, unless the condition is specifically looked for. The considerable frequency of this SNP in the studied Egyptian cohort, and the prevalence of environmental triggers that tend to over-express it as iron deficiency [76, 132] and dietary phosphate overconsumption [126], should generate an impetus to develop nationwide gene studies to further explore the role of genetic factors in such significant health problems. Increased serum iFGF23 has emerged as an early biochemical marker of increased risk for hypertension and hypertensive CKD in genetically predisposed individuals. Further research should determine its practical utility as a diagnostic parameter, and possibly as a therapeutic target, and should try to define its recommended target range that provides the best balance between its adaptive and maladaptive effects in patients with different stages of CKD.

To the best of our knowledge, this is the first study to address the rs7955866 *FGF23* SNP in the Arab region. Studies of this SNP are generally scarce. We acknowledge the limitations imposed by the limited number of included subjects and the cross-sectional nature of the study, which undermine its power to draw firm conclusions about cause–effect relationships, particularly regarding very complex and multi-factorial traits like hypertension and CKD. Hypertension load may have been better assessed by ambulatory BP monitoring [133]. The study did not include other *FGF23* genetic variants or other related biochemical measures like urinary albumin and phosphate excretion, active vitamin D, and markers of iron profile.

Conclusions

For the first time in an Egyptian cohort, we report a relatively high frequency of the rs7955866 SNP. It may remain dormant or become upregulated in response to some environmental triggers, notably dietary phosphorus excess, leading to increased circulating iFGF23 with ensuing hypertension and/or renal impairment. Subjects with this SNP, particularly in the homozygous form, are at increased risk for CKD of presumably "unknown" etiology, with a tendency for early onset hypertension and increased circulating iFGF23 out of proportion with the degree of renal impairment.

Large-scale population studies are needed to confirm these findings and explore the role of blockers of the renin–angiotensin–aldosterone system and sodium chloride cotransporters in mitigating hypertension associated with FGF23 excess.

Supplementary Information

Additional file 1. Three excel sheets displaying further results analysis.

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Declarations

Ethics approval and consent to participate
This study was carried out in accordance with the tenets of the Declaration of Helsinki and its later amendments for experiments involving humans. It was approved by the Medical Research Ethics Committee of the Medical Research Institute, Alexandria University. Written informed consents were obtained from all participants; and their identification information was kept confidential.

Consent for publication
The authors certify that each author has participated sufficiently in this research and takes full responsibility for the validity of the entire study.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Internal Medicine Department, Medical Research Institute, Alexandria University, Alexandria, Egypt. [2]Chemical Pathology Department, Medical Research Institute, Alexandria University, Alexandria, Egypt. [3]Medical Biochemistry Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt.

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