10.4274/jcrpe.galenos.2023.2023-3-11

Case report

Sotos Syndrome and Nephrocalcinosis, a Rare But Possible Association due to Impact on Contiguous Genes

González-Rodríguez JD et al. Sotos Syndrome and Nephrocalcinosis

Juan D. González-Rodríguez[1], Esther Q. Inglés-Torres[1], José E. Cabrera-Sevilla[1], Salvador Ibáñez-Micó[2], Francisca Bermejo-Costa[3], Ascensión Vera-Carbonell[4], Juan A. Bafalliu-Vidal[4], Pedro Cortés-Mora[3], Ana Lorente-Nicolás[5], José María Donate-Legaz[6].
[1]Pediatric Nephrology Unit. Santa Lucia General University Hospital, Cartagena, Murcia, Spain.
[2]Pediatric Neurology Unit. Virgen de la Arrixaca Clinical University Hospital, Murcia, Spain.
[3]Pediatric Gastroenterology Unit. Santa Lucia General University Hospital, Cartagena, Murcia, Spain.
[4]Biochemistry and Clinical Genetics Center. Virgen de la Arrixaca Clinical University Hospital, Murcia, Spain.
[5]Pediatric Cardiology Unit. Santa Lucia General University Hospital, Cartagena, Murcia, Spain.
[6]Pediatric Endocrinology Unit. Santa Lucia General University Hospital, Cartagena, Murcia, Spain

What is already known on this topic? What this study adds?
Up to 15% of patients with Sotos Syndrome present renal disorders, the most common of which is vesicoureteral reflux.
However, nephrocalcinosis is a less common clinical disorder reported only in cases of Sotos Syndrome due to deletion that includes *SLC34A* and other genes, associated with phosphate wasting and hypercalciuria.
In these patients, delayed growth and impaired kidney function are possible and long-term follow-up is recommended.

Abstract
One-month old breastfeeding infant, full-term birth, with normal anthropometric measurements at birth is referred to Pediatric Nephrology due to a nephrocalcinosis. The patient presents dysmorphic features and heart disease. A metabolic study is conducted on blood and urine yielding results within normal parameters, except for renal concentration test and acidification test. At 6 months of age, patient presents overgrowth, which along with other clinical signs arouse suspicion of Sotos Syndrome. Molecular genetic testing detects heterozygous deletion in 5q35 between bands q35.2 and q35.3, affecting genes *NSD1, SLC34A1* and *FGFR4*, which is compatible with this syndrome and with nephrocalcinosis as a rare association.
Keywords: Sotos syndrome, nephrocalcinosis, *NSD1*, *SCL34A1, FGFR4*, case report

Juan David González Rodríguez, Nefrología Pediátrica HGU Santa Lucía, Servicio de Pediatría Complejo Hospitalario Universitario de Cartagena, C/ Mezquita, s/n, Paraje Los Arcos, 30202, Santa Lucía. Cartagena (Murcia) - Spain
+00 34 968110745
juandavid.gonzalez@um.es
18.04.2023
03.07.2023

Published: 09.08.2023

Introduction
Sotos Syndrome is a dominant autosomal hereditary disease with a prevalence of approximately 1 in 14,000 newborns[1,2]. It is characterized by overgrowth, a distinctive facial phenotype and learning disabilities[3]. Actually, it is a multisystemic disorder and up to 15 % of patients present renal disorders, the most common of which is vesicoureteral reflux. Most cases present point mutation in gene *NSD1*; a de novo mutation in over 95 % of cases. Clinical findings in European patients show 10-15 % microdeletion of 5q35 affecting this gene, as detected in the clinical case discussed herein[2]. The exceptional circumstance in this patient is the presentation of genetically confirmed Sotos Syndrome in association with a less common clinical disorder, nephrocalcinosis, which can be explained by the alteration of contiguous genes included in the deletion found.

Clinical case presentation
37-day old male nursing infant, referred to Pediatric Nephrology due to a finding of bilateral medullary nephrocalcinosis in an ultrasound conducted at one month of life. The patient was born from a controlled pregnancy without incident, with normal prenatal echographic controls, from non-consanguineal parents and with no family history that is relevant to the case. Labor at 42 weeks of pregnancy, concluded by C-section due to failed induction, with adequate weight at birth (3,900 g +0.93 from standard deviation [SD]). Patient is admitted to the Neonatal Unit at 3 hours of life due to hypoglucemia, developing histologically confirmed eosinophilic colitis symptoms at 8 days of life and presents good progress after 10 days of parenteral feeding. During his hospital stay, the patient received aminoglycoside treatment.
The infant is monitored in Pediatric Cardiology due to *ostium secundum* atrial septal defect without hemodynamic compromise, patent ductus arteriosum and mild pulmonary valvular stenosis, and in Pediatric Neurology due to mild hypotonia and subtle dysmorphic features (broad forehead, lack of frontotemporal hair, long and narrow face, single transverse palmar crease, scaphocephaly and anteverted nares), presenting a front left paraventericular ependymal cyst and FLAIR hyperintensity in the corona radiata in brain magnetic resonance imaging.
Stimulation is started in Early Care due to mild neurodevelopmental retardation. Neonatal metabolic screening is compatible with normal characteristics and otoacoustic emissions yield negative results. Patient receives a daily supplement of vitamin D3 (400 IU) and omeprazole. Normal ophtalmological examination.
Given the presence of bilateral nephrocalcinosis and the patient's history, the study is started with a proposal for an initial differential diagnosis involving the context of the syndrome, a renal condition and/or a multi-factor etiology due to associated neonatal comorbidity. A metabolic study is conducted on blood and urine with normal results, including thyroid hormones, lactate, pyruvate and organic acids, although a gradual decrease of blood phosphate levels is observed, reaching values even lower than the reference values for the patient's age, with phosphate reabsorption and normal fibroblast growth factor 23 (FGF23) values inappropriate for phosphatemia, along with an increase of 1,25 $(OH)_2$ vitamin D3 and parathyroid hormone (PTH) in low-normal range, appropriate for serum calcium levels, within the upper end of normalcy *(Table 1)*. Normal proteinuria results, including tubular proteinuria. Initially, renal function shows increase of cystatin C and NAG values and abnormal renal concentration test results after stimulation with desmopresin, reaching maximum urinary osmolality of 307 mOsm/Kg (normal value > 562 mOsm/Kg), as well as altered renal acidification capacity, reaching a maximum pCO2 of 54 mmHg after stimulation with bicarbonate and acetazolamide (normal value > 70 mmHg).

At four months of age, patient presents *Escherichia Coli* urinary tract infection with fever, with normal cystourethrogram, while the renal gammagraphy shows right kidney hypodysplasia with no associated cortical lesions. At six months, overgrowth is detected: weight +2.50 SD, height +2.63 SD and head circumference +2.38 SD. However, the anthropometric values gradually return to normal; bone age corresponds to patient's age and no associated skeletal disorders are observed.

Sotos Syndrome is suspected due to clinical observations of dysmorphic syndrome, overgrowth and heart disease; on association of nephrocalcinosis, an expanded genetic study is requested to search for microdeletions that affect genes other than gene *NSD1* due to contiguity. The FISH test reveals absence of hybridization signal in one of the locus of Sotos Syndrome in chromosome 5, with a normal pattern in the parents (*Figure 1*). Additionally, oligo-array CGH confirmed de novo deletion of approximately 2 Mb in 5q35.2q35.3 (from 175,580,042 to 177,386,153 bp) in the patient, as oligo-array CGH using maternal versus paternal DNA yielded a normal hybridization profile, including genes *SLC34A1 and FGFR4* in this range, among others. (*Figure 2*)

At 4 years, oral phosphate was prescribed, with poor tolerance, so he finally began thiazides due to improvement in serum calcium levels. In the last assessment at 7 years of age, weight and height are -0.07 SD and +0,44 SD, respectively, with normal growth rate (-0.7 SD) and IGF-1 and IGFBP3 values within a normal range for the patient's age. Bilateral medullary nephrocalcinosis persists, with renal asymmetry (right kidney, 50-75th percentile and left kidney > 95th percentile), and the abnormal test results described in Table 1 above, along with mild hypercalciuria, although there is an improvement in phosphate levels and normalization of renal function tests. Cardiological evaluation shows continuation of mild patent ductus arteriosum without other findings. From a neurological point of view, the patient is still receiving cognitive and communication stimulation and psychomotor retardation is improving; cow's milk protein challenge has been started and tolerance is good.

Discussion:

The *NSD1* gene is the only gene currently known to cause Sotos Syndrome. Among European patients with typical findings of this syndrome, up to 15 % present 5q35 microdeletion that affects that gene, and associated disorders may appear when these deletions affect other genes[2,4]. There is no genotype-phenotype correlation in this syndrome, but in cases due to 5q35 microdeletion, overgrowth is less obvious and usually has more neurological impact, as is the case in our patient, while nephrocalcinosis is a phenotypic characteristic reported only in cases of Sotos Syndrome due to microdeletion[4].

The literature contains very few cases of nephrocalcinosis in Sotos Syndrome patients. Saugier-Veber *et al*, 2007, published three cases due to deletion with associated nephrocalcinosis, suggesting a genetic predisposition in the deletion area[5]. Kenny *et al*, 2011, presented two pediatric cases of 5q35 chromosome microdeletion that reaches genes *NSD1* and *SLC34A1*, explaining the wider phenotypic spectrum of this syndrome[6]. This last gene encodes an important renal phosphate carrier (NaPi-IIa) and mutations thereof have been associated with Nephrolithiasis/osteoporosis, hypophosphatemic 1 (OMIM#612286), Fanconi reno-tubular syndrome 2 (OMIM#613388) and Hypercalcemia infantile 2 (OMIM# #616963). Recessive mutations of this gene have been associated with Fanconi Syndrome and hypophosphatemic rickets, as well as familial cases of hypophosphatemia and nephrocalcinosis[7,8]. On the other hand, heterozygous mutations of this gene in patients with nephrolithiasis and osteoporosis have been published[9,10]. Moreover Schlingmann *et al*, 2016, described a homozygous mutation in the same gene as a cause for idiopathic hypercalcemia in familial and sporadic cases, explaining the hypercalcemia with the suppression of FGF23 caused by hypophosphatemia, caused in turn by inactivation of the renal phosphate carrier NaPi-IIa[11]. Their results suggested that supplementation with oral phosphate could help correct calcium metabolism in patients with *SLC34A1* mutation, although this treatment was not tolerated in our patient.

More recently, overlapping phenotypes associated with *SLC34A1*, *SLC34A3* and *CYP24A1* mutations have been described, and that not all the patients showed improvements in hypercalciuria and nephrocalcinosis, despite improvement in hypercalcemia and 1,25 $(OH)_2$ vitamin D3 levels, as has happened in our case.[12,13] Moreover, an attenuation of renal phosphate wasting with advancing age has been observed, which may reflect the decreasing importance of NaPi-IIa for phosphate homeostasis over time, and other studies found impaired kidney function at a mean age of 23.8 years, even in subjects with a heterozygous mutation, suggesting the need for long-term follow-up of these patients.[14,15] Mutsaers *et al*, 2014, described a case of Sotos Syndrome due to 5q35 microdeletion with impact on gene *NSD1* and FGF receptor gene 4 (*FGFR4*), also included in the affected range of our patient, presenting transient hypercalcemia but no nephrocalcinosis[16]. This study proves the existence of a change in the molecular profile for the expression of FGF receptors (FGFR) during human renal development and that the expression of FGFR4 decreases with age. The authors propose that the heterozygous microdeletion detected causes inactivation of this FGF23 receptor, causing damaged signaling at an early stage of development, thus affecting calcium homeostasis, mainly mediated by FGF23 binding to FGFR3 and FGFR4. As a response to elevated calcemia, osteocytes increase FGF23 release under normal conditions. Its purpose includes decreasing 1,25 $(OH)_2$ vitamin D3 and the levels of PTH to achieve a negative balance of calcium, which cannot be achieved when this signaling pathway is damaged.

Considering the complex regulation mechanisms of mineral metabolism and taking into account the studies published and the analytical results of our patient, which show normal intact FGF23 values, we think that the damaged signaling pathway caused by the alteration of gene *FGFR4* may activate 1,25 $(OH)_2$ vitamin D3, thus favoring the appearance of hypercalcemia and hypercalciuria. In addition, this alteration of *FGFR4* could also contribute to the inappropriately normal FGF23 level, which may in turn inhibit the expression of renal carriers NaPi of the proximal tubule through its binding mainly to FGFR1 and thus act as an additional stimulus for the renal loss of phosphate and the hypophosphatemia of the patient, already boosted by the inactivation of carrier NaPi-IIa due to the mutation of *SLC34A1*. Although hypophosphatemia is not very significant, due to the heterozygous mutation of gene and that this defect could be partially compensated by the NaPi-IIc contransporter, it is another major stimulus for the increase of 1,25 $(OH)_2$ vitamin D3[17,18]. On the other hand, the normalization of cystatin C, NAG values and renal water handling suggest a relationship with neonatal kidney injury and aminoglycoside treatment. *(Figure 3)*

Lastly, our patient's growth decreased in the last period after an initial increase, possibly explained by the various alterations of genes, as delayed growth in hypophosphatemic syndromes is common, although not constant and variable, and the regulatory action of FGF23 on bone mineralization is currently known[19-20].

In conclusion, nephrocalcinosis is a phenotypic characteristic reported in cases of Sotos Syndrome due to deletion that includes *SLC34A* and other genes, associated with phosphate wasting, hypercalcemia, hypercalciuria and elevated 1,25 $(OH)_2$ vitamin D3 levels. In these patients, is recommended a long-term follow-up due to the risk of impaired kidney function, although future studies will have to establish the most appropriate treatment.

Funding: This study was supported by FORIIN Foundation, Cartagena (Spain)

References

1. Nagai T, Matsumoto N, Kurotaki N, Harada N, Niikawa N, et al. Sotos syndrome and haploinsufficiency of NSD1: clinical features of intragenic mutations and submicroscopic deletions. J Med Genet. 2003; 40:285–289.
2. Tatton-Brown K1, Douglas J, Coleman K, Baujat G, Chandler K, et al. Childhood Overgrowth Collaboration. Multiple mechanisms are implicated in the generation of 5q35 microdeletions in Sotos syndrome. J Med Genet. 2005; 42:307-13.
3. Edmondsonc AC, Jennifer M. Kalish JM. Genetic Advances in Intellectual Disability. Overgrowth Syndromes. J Pediatr Genet. 2015; 4:136–143.
4. Tatton-Brown K, Douglas J, Coleman K, Baujat G, Cole TRP, et al. Genotype-Phenotype Associations in Sotos Syndrome: An Analysis of 266 Individuals with NSD1 Aberrations. Am J Hum Genet. 2005; 77(2): 193–204.

5. Saugier-Veber P, Bonnet C, Afenjar A, Drouin-Garraud V, Coubes C et al. Heterogeneity of NSD1 alterations in 116 patients with Sotos syndrome. Hum Mutat. 2007; 28:1098–1107.
6. Kenny J, Lees MM, Drury S, Barnicoat A, van't Hoff W, et al. Sotos syndrome, infantile hypercalcemia, and nephrocalcinosis: a contiguous gene syndrome. Pediatr Nephrol. 2011, 26:1331-1334
7. Magen D, Berger L, Coady MJ, Ilivitzki A, Militianu D, et al. A loss-of-function mutation in NaPi-IIa and renal Fanconi's syndrome. N Engl J Med. 2010, 362:1102–1109
8. Rajagopal A, Braslavsky D, Lu JT, Kleppe S, Clément F, et al. Exome sequencing identifies a novel homozygous mutation in the phosphate transporter SLC34A1 in hypophosphatemia and nephrocalcinosis. J Clin Endocrinol Metab. 2014; 99:2451-2456.
9. Prie D, Huart V, Bakouh N, Planelles G, Dellis O, et al. Nephrolithiasis and osteoporosis associated with hypophosphatemia caused by mutations in the type 2a sodium-phosphate cotransporter. N Engl J Med. 2002, 347:983–991.
10. Wagner CA, Rubio-Aliaga I, Hernando N. Renal phosphate handling and inherited disorders of phosphate reabsorption: an update. Pediatr Nephrol. 2019 Apr;34(4):549-559
11. Schlingmann KP, Ruminska J, Kaufmann M, Dursun I, Patti M, et al. Autosomal-Recessive Mutations in SLC34A1 Encoding Sodium-Phosphate Cotransporter 2A Cause Idiopathic Infantile Hypercalcemia. J Am Soc Nephrol. 2016; 27: 604–614.
12. Molin A, Lemoine S, Kaufmann M, Breton P, Nowoczyn M, Ballandonne C, et al. Overlapping Phenotypes Associated With CYP24A1, SLC34A1, and SLC34A3 Mutations: A Cohort Study of Patients With Hypersensitivity to Vitamin D. Front Endocrinol (Lausanne). 2021 Oct 13;12:736240.
13. Gurevich E, Levi S, Borovitz Y, Alfandary H, Ganon L, Dinour D, et al. Childhood Hypercalciuric Hypercalcemia With Elevated Vitamin D and Suppressed Parathyroid Hormone: Long-Term Follow Up. Front Pediatr. 2021 Nov 10;9:752312.
14. Gordon RJ, Li D, Doyle D, Zaritsky J, Levine MA. Digenic Heterozygous Mutations in SLC34A3 and SLC34A1 Cause Dominant Hypophosphatemic Rickets with Hypercalciuria. J Clin Endocrinol Metab. 2020 Jul 1;105(7):2392–400.
15. Janiec A, Halat-Wolska P, Obrycki Ł, Ciara E, Wójcik M, Płudowski P, et al. Long-term outcome of the survivors of infantile hypercalcaemia with CYP24A1 and SLC34A1 mutations. Nephrol Dial Transplant. 2021 Jul 23;36(8):1484-1492.
16. Mutsaers HAM, Levtchenko EN, Martinerie L, Pertijs JCLM, Allegaert K, et al. Switch in FGFR3 and -4 expression profile during human renal development may account for transient hypercalcemia in patients with Sotos syndrome due to 5q35 microdeletions. J Clin Endocrinol Metab. 2014, 99:E1361–E1367.
17. Blaine J, Chonchol M, Levi M. Renal control of calcium, phosphate, and magnesium homeostasis. Clin J Am Soc Nephrol. 2015;10(7):1257-72.
18. Bacchetta J, Bardet C, Prié D. Physiology of FGF23 and overview of genetic diseases associated with renal phosphate wasting. Metabolism. 2020 Feb;103S:153865. doi: 10.1016/j.metabol.2019.01.006. Epub 2019 Jan 19. PMID: 30664852.
19. Santos F, Fuente R, Mejia N, Mantecon L, Gil-Peña H, et al. Hypophosphatemia and growth. Pediatr Nephrol. 2013; 28:595-603.
20. Guo YC, Yuan Q. Fibroblast growth factor 23 and bone mineralisation. Int J Oral Sci. 2015;7(1):8-13.

Table 1. Progressed analytical data of the patient.

Age	1 m	2 y	4 y	6 y	7 y
Plasma *(reference values in parentheses)*					
Cr [mg/dl] *(0.32-0.59)*	0.34	0.34	0.46	0.59	0.55
Cystatin C [mg/L] *(0.62-1.11)*	2	0.62	1.02	0.96	-
Urate [mg/dl] *(2.2-4.5)*	2.4	2.7	-	3.1	2.6
Ion Ca [mg/dl] *(4.6-5.3)*	5.8	5.5	5	5.3	-
Phosphate [mg/dl] *(4.1-5.9)*	5.3	3.9	3.9	3.2	4
Magnesium [mg/dl] *(1.6-2.6)*	2.1	2.0	1.8	1.8	1.8
AP (U/L) *(142-335)*	500	335	302	379	319
PTH [pg/ml] *(11-60)*	14	24	14	15	14
25 (OH) VitD3 [ng/ml] *(17-49)*	-	42	39	33	39
$1,25(OH)_2$ VitD3 [pg/ml] *(45-102)*	-	249	-	99	-
Intact FGF23 [pg/ml] *(36±18)*	-	51.3	-	-	-
pH_p *(7.35-7.45)*	-	7.37	7.39	7.37	7.38
Bicarbonate (mEq/L) *(20-26)*	-	25	23	22	23
Urine *(reference values in parentheses)*					
pH_u	7.5	7	7	7	7.5
Ca/Cr [mg/mg] *(< 0.20-0.28)*	0.52	0.35	0.28	0.26	0.25
Citrate/Cr [mg/g] *(> 250-420)*	706	687	307	172	314
Ca/citrate [mg/mg] *(< 0.33)*	0.74	0.51	0.92	1.49	0.58
Oxalate/Cr (mg/g) *(< 110)*	130	60	50	-	41
Prot/Cr [mg/mg] *(< 0.2)*	0.68	0.18	0.18	0.11	0.11
NAG/Cr [U/g] *(< 6-11)*	150	15	-	3	3
TRP (ml/dl GFR) *(91.05±4.71)*	91	90	79	80	88
TP/GFR [mg/dl] *(4.6±0.6)*	4.8	3.62	3.1	2.3	3.5
V/GFR (ml/dl GFR) *(0.59±0.22)*	1.44	1.10	1.20	0.91	0.98

M: month. Y: years. Cr: creatinine. *Ca:* calcium. *AP:* alkaline phosphatase. *PTH:* parathyroid hormone. *25(OH)vitD3:* 25-hydroxyvitamin D3. *1,25(OH)2vitD3:* 1,25-dihydroxyvitamin D3. *pHp:* plasma pH. *pHu:* urine pH. *Prot/Cr:* protein/creatinine ratio. *TRP:* tubular reabsorption of phosphate. *TP/GFR:* tubular reabsorption of phosphate per dl of glomerular filtrate. *V/GFR:* urinary volume per dl of glomerular filtrate.

Figure 1. A.- FISH of the patient with probe combined for the loci of de Cri-du-chat (*UBE2QL1*, 5p15.31; *CTNND2*, 5p15.2) and Sotos (*NSD1*, 5q35) Syndromes. The signals in 5p15 are present in both chromosome 5 pairs. The arrow shows lack of hybridization of the *NSD1* clone (green) in one of the chromosome 5 pairs. B and C.- FISH of the father and mother, respectively, with the same probe. Both chromosome 5 pairs show signals both in 5p15 and 5q35

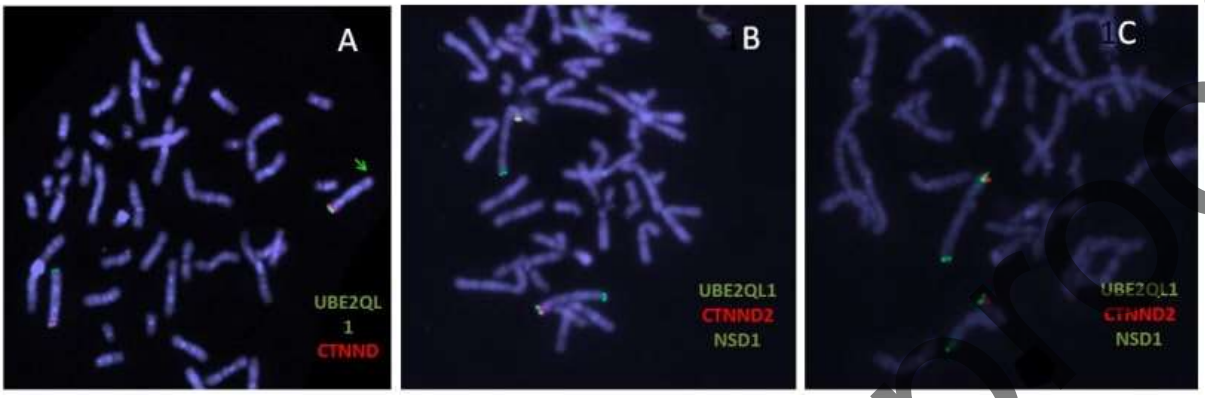

Figure 2. A.- Oligo-array CGH of the patient, showing an enlargement of the deleted region (red bar) in chromosoma 5. B.- Oligo-array CGH of the same region in chromosome 5 after comparing the DNA of the parents, illustrating a normal result

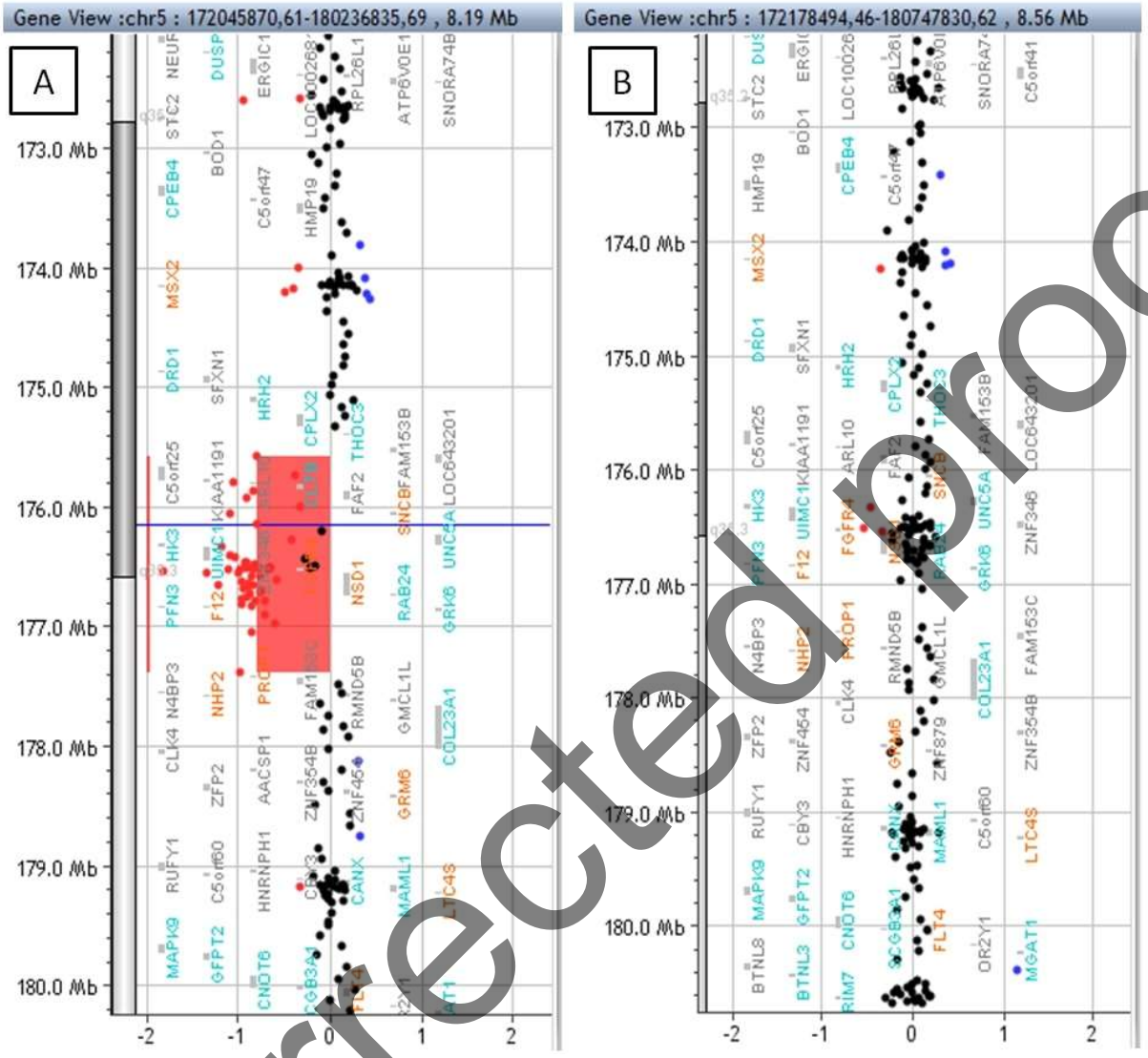

Figure 3. The damaged signaling pathway due to an alteration of gene *FGFR4* could cause an activation of PTH and 1,25 (OH)2 vitamin D3, due to stimulus of enzyme 1α hydroxylase and the lowered expression of 24 hydroxylase, thus favoring hypercalcemia and hypercalciuria. In addition, this alteration of *FGFR4* may also contribute to the inappropriately normal FGF23 level, which through binding to FGFR1 would decrease tubular reabsorption of phosphorus into the proximal tubule, exacerbating the hypophosphatemia associated with renal loss due to the inactivation of the NaPi-IIa carrier. Hypophosphatemia would be another major stimulus for the increase of 1,25 (OH)2 vitamin D3, although with a negative impact on the production of FGF23 and PTH