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Study of LIF polymorphisms and phenotypes of CAKUT in a Brazilian pediatric population

Estudo de polimorfismos LIF e fenótipos de CAKUT em uma população pediátrica brasileira

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ABSTRACT

The congenital anomalies of the urinary tract — CAKUT emerge from the interaction between genetic anomalies and environmental factors present before and during pregnancy. The aim of this study was to evaluate possible associations between pathways of gene polymorphism LIF and CAKUT. The study was done with 538 Brazilian volunteers, the control group being 160 females and 102 males, totaling 262 healthy individuals. The case group contained 115 females and 161 males, totaling 276 pediatric patients originated from the CAKUT ambulatory from Federal University of Minas Gerais, Brazil. The rs 737812, 929271 and 737921 of LIF were investigated. In association analyzes between cases and controls, no correlation was seen between the rs 737812 and 929271 of LIF and CAKUT. There was a positive association between rs 737921 and general CAKUT as well as with the various phenotypes studied except of hydronephrosis and of multicystic renal dysplasia. Force of association of rs LIF to general CAKUT is measured by a p-value of 0.0009 after 1000 permutations.
Keywords: Congenital anomalies of the urinary tract (CAKUT); LIF; Gene candidate; Association studies.

RESUMO

As anomalias congênitas do trato urinário – CAKUT surgem da interação entre anomalias genéticas e fatores ambientais presentes antes e durante a gravidez. O objetivo deste estudo foi avaliar possíveis associações entre as vias do polimorfismo gênico LIF e CAKUT. O estudo foi feito com 538 voluntários brasileiros, sendo o grupo controle 160 mulheres e 102 homens, totalizando 262 indivíduos saudáveis. O grupo de casos continha 115 mulheres e 161 homens, totalizando 276 pacientes pediátricos oriundos do ambulatório CAKUT da Universidade Federal de Minas Gerais, Brasil. Foram investigados os rs 737812, 929271 e 737921 da LIF. Nas análises de associação entre casos e controles, não foi observada correlação entre as rs 737812 e 929271 de LIF e CAKUT. Houve associação positiva entre rs 737921 e CAKUT geral, bem como com os vários fenótipos estudados, exceto hidronefrose e displasia renal multicística. A força de associação de rs LIF ao CAKUT geral é medida por um valor p de 0,0009 após 1000 permutações.

Palavras-chave: Anomalias congênitas do trato urinário (CAKUT); LIF; Gene candidato; Estudos de associação.

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INTRODUCTION

The Congenital Abnormalities of the Kidney and Urinary Tract (CAKUT) are frequent causes of renal chronic diseases (RCD) in the childhood. The main known phenotypes are hydronephrosis, renal agenesis, renal hypo-dysplasia, multicystic kidney dysplasia, duplex renal collecting system, ureteropelvic junction obstruction, megaureter, posterior urethral valves (PUV) and vesicoureteral reflux (VUR). Although these abnormalities are often asymptomatic and undiagnosed, their social and individual costs are weighed. Studying different populations, researchers found that CAKUT is related approximately with 1/3 to 2/3 of cases of childhood-onset of chronic kidney disease (CKD) (HARAMBAT; VAN STRALEN; KIM, 2012). In some populations, studies refer that 20% to 50% of all fetal congenital anomalies are CAKUT (MELO; AGUIAR; BOUZADA, 2012; MIZUNO, 2010).

CAKUT arises from genetic abnormalities and environmental factors present before and during pregnancy. Among the environmental factors are intrauterine conditions such as maternal diabetes, maternal diet and drug use during pregnancy. Knowledge of the genetic alterations comes from the study of candidate genes identified in family studies, from the consideration of important genes during nephrogenesis and from the identification of genes causing CAKUT syndromic forms in animal models and in genes known as deregulators of human CAKUT (RENKEMA et al., 2011).

The normal embryogenesis consists of events ranging from the emergence of pronefro, at the fourth week of pregnancy, until the formation of the definitive kidney. The main event in this process is the interrelation between the ureteric bud with the metanephric blastema. During the first four weeks of gestation, producing mesenchymal cells IGFII are able to trigger the cascade of events that leads to the development of metanephro. In the fourth week, the bud sends message to the blastema via FGF2 which activates the process of condensation.

Before ureteric bud breaks into the blastema it sends messages to mesenchymal cells via FGF2 and starts the process of condensation. Mesenchymal cells produce BMP4 that line around the Wolfian duct in locations outside the legitimate site where ureteric bud would form. Cells expressing AT2 adjacent to the legitimate site undergo apoptosis and allow GDNF reach the site of the ureteric bud induction. With WT1 expression of the mesenchymal cells begin to agglomerate and there is suppression of IGFII. GDNF/

GDNFα/RET becomes activated and in turn activates the WNT11. WNT11 is involved in bud formation and its divisions.

PAX2 is another important gene whose role is inducing differentiation of mesenchymal cells into epithelial cells. WT4 is also an important indicator and transcriptor involved with guiding of the tubular nephron development. There are numerous other genes active in the nephrogenesis cascade. This study aims to evaluate the eventual association of LIF - Leukemia inhibitory factor with CAKUT considering that it is an important regulator of self renewal of pluripotent cells. This gene has been described as stimulating the expression WNT4, BCL2 and BMP4 which inhibit apoptosis of epithelial and pre-epithelial cells in nephrogenesis. It is well known that a change in any of the organogenesis pathways can lead to wide spectrum of CAKUT. The investigation of the causes of CAKUT is essential for proper stratification and disease prognosis prediction.

PATIENTS AND METHODS

A. Cases Group

This study included 276 pediatric patients (115 females and 161 males) diagnosed with antenatal renal and/or urinary tract alterations followed up at the Pediatric Nephrology Unit of Federal University of Minas Gerais (Brazil) from 2009 to 2014, whose parents gave their consent to participate in the study protocol.

B. Control Group

The control group included 266 healthy sex- and age-matched subjects from the same geographic area of the patients. The controls were randomly invited to participate of the study. All controls were normotensive without family history of renal chronic diseases or CAKUT. Healthy status was also determined through the medical history and either a parental report or self-report to rule out the presence of chronic or acute diseases.

C. Ethical aspects

CAKUT patients and healthy controls, both represented by their parents, gave consent and assented to participate in this study. The study protocol is very strict to avoid any possibility of interference with medical recommendations or prescriptions.

D. Blood Collection and DNA Extraction

After informed consent, all cases and controls were submitted to an intravenous puncture to collect 10 ml of peripheral blood samples. DNA was extracted from peripheral blood lymphocytes in accordance with the method described by Lahiri and Nurnberger (2011).

E. Genotyping and allelic discrimination

The made-to-order TaqMan™ (Applied Biosystems) probes to rs929271, rs737812, and rs737921 2077642 were used with 50 ng of DNA of each sample. Allelic discrimination analysis was performed in plates of 96 wells. A real-time PCR device (Mx3005PTMStratagene, GE Healthcare Life Sciences) was used according to the following protocol: samples were exposed to 95 ºC for 10 min, then 50 cycles at 95 ºC for 15 s, and at 60 ºC for 1 min. Case and control samples were randomly arranged in well plates with at least 20% of genotypes retyped as quality control. The three SNPs used were chosen in the HapMap database with a selection criterion of $r^2 > 0.8$ and $MAF > 0.15$. Table 1 shows the position of each LIF SNPs selected for this study.

Table 1. Location of the LIF gene SNP used in this study

SNO code	Gene location	Mutation	Ancestral alelle	MAF	Cromossome position
737812	3' UTR	A/C	C	0,261	22:30243121
929271	3' UTR	G/T	T	0,300	22:30242237
737921	Intron	A/G	G	0,151	22:30244225

F. Statistical Analysis

Statistical analysis was performed using UNPHASED version 3.1.4 (Frank Dudbridge, MRC Biostatistics Unit, Cambridge, UK, 2008) with 1,000 permutations. Differences between genotype distribution and allele frequency were tested by Chi-square analysis. A p-value ≤ 0.05 was considered statistically significant. HAPLOVIEW version 4.2 searched for linkage disequilibrium and analyzed the haplotype maps according to Barrett et al. (2005). The Hardy Weinberg Equilibrium (HWE) was tested by HAPLOVIEW being found the χ^2 values of Table 2. It is seen that the sample presents a very light HW disequilibrium for the rs 737921. This will be commented in the section 4.

G. Frequency analysis

UNPHASED analyses of the study sample didn't show significant differences between cases and controls alleles and genotype frequencies for rs929271 and rs737812. The same conclusion was found when the various CAKUT phenotypes were considered. For rs737921, UNPHASED analysis showed statistically significant differences in allele and genotype frequencies between cases and controls. See Tables 3-8.

Table 2. HWE of LIF tagSNPs.

TagSNP		Cases and controls	
		p value	χ^2
LIF	rs 929271	0,2084	0,4296
	rs 737812	0,5485	0,4753
	rs 737921	**0,0424**	0,2864

RESULTS

The sample included 542 individuals: 276 pediatric patients (115 females and 161 males) diagnosed with antenatal renal and/or urinary tract alterations and 266 healthy sex- and age-matched subjects from the same geographic area of the patients. Idiopathic hydronephrosis was the most common postnatal finding in our patients with pre-natal RDP higher than 5 mm. However, when an uropathy was diagnosed, the most common phenotypes were on this order: VUR, UPJO, MCDK and PUV.

The prevalence of LIF polymorphisms for the SNPs rs 929271, rs 737812, and rs 737921 in CAKUT patients was compared with controls (Tables 3). No statistical differences in allele and genotype frequencies were observed for the markers rs 929271 and rs 737812. At the SNP rs 737921, the genotype AA was significantly increased in CAKUT group, while the GG was higher in control group. The frequencies of LIF polymorphisms for the marker rs 929271, rs 737812 and rs 737921 were also analyzed in the most frequent CAKUT phenotypes (Hydronephrosis, VUR, UPJO, MCDK and PUV) and compared with controls on Tables 4,5,6,7,8.

Table 3. Allelic and genotypic frequencies for CAKUT.

		Frequencies					
SNP LIF	**All.**	**Cases N (%)**	**Controls N (%)**	***χ² (df)***	***p***	***P+***	***OR (95%CI)***
rs929271	T	382 (71.54)	366 (71.76)	0.006	0.9345	NA	1.00 (ref.)
	G*	152 (28.46)	144 (28.24)	(1)			1.01 (0.77-1.32)
	TT	140 (52.43)	134 (52.55)		0.9906	NA	1.00 (ref.)
	TG	102 (38.20)	98 (38.43)	0.018			0.99 (0.69-1.43)
	GG	25 (9.36)	23 (9.02)	(2)			1.04 (0.56-1.92)
rs737812	C	391 (73.50)	366 (73.20)	0.01	0.9143	NA	1.00 (ref.)
	A*	141 (26.50)	134 (26.80)	(1)			0.98 (0.74-1.29)
	CC	142 (53.38)	133 (53.20)		0.9825	NA	1.00 (ref.)
	CA	107 (40.23)	100 (40.00)	0.03			1.00 (0.69-1.43)
	AA	17 (06.39)	17 (06.80)	(2)			0.93 (0.45-1.91)
rs737921	G	332 (62.64)	385 (75.20)	19.24	**1.1485**	**0.09**	1.00 (ref.)
	A*	198 (37.36)	127 (24.80)	(1)	**7e-005**		1.80 (1.38-2.36)
	GG	98 (36.98)	138 (53.91)			**0.09**	1.00 (ref.)
	GA	136 (51.32)	109 (42.58)	22.43	**1.3421**		1.75 (1.22-2.52)
	AA	31 (11.70)	9 (03.51)	(2)	**9e-005**		4.85 (2.21-10.6)

*: Polymorphic allele; P+: p value corrected for 1,000 permutations

Table 4. Allelic and genotypic frequencies for hydronephrosis (H).

		Frequencies					
SNP LIF	**All.**	**H N (%)**	**Controls N (%)**	***χ² (df)***	***p***	***P+***	***OR (95%CI)***
rs929271	T	111 (72.08)	356 (71.77)	0.0054	0.9416	NA	1.00 (ref.)
	G*	43 (27.92)	140 (28.23)	(1)			0.98 (0.66-1.47)
	TT	41 (53.25)	130 (52.42)		0.9862	NA	1.00 (ref.)
	TG	29 (37.66)	96 (38.71)	0.027			0.95(0.55-0.65)
	GG	7 (09.09)	22 (08.87)	(2)			1.00(0.40-2.53)
rs737812	C	119 (76.28)	360 (74.07)	0.3070	0.5795	NA	1.00 (ref.)
	A*	37 (23.72)	126 (25.93)	(1)			0.89 (0.58-1.35)
	CC	46 (58.97)	131 (53.91)		0.6636	NA	1.00 (ref.)
	CA	27 (34.62)	98 (40.33)	0.82 (2)			0.78 (0.45-1.35)
	AA	5 (06.41)	14 (05.76)				1.01 (0.34-2.98)
rs737921	G	105 (68.18)	384 (76.49)	4.15 (1)	0.0416	0.1029	1.00 (ref.)
	A*	49 (31.82)	118 (23.51)				0.76 (0.40-1.45)
	GG	35 (45.45)		6.82 (2)	**0.0328**	**0.1149**	1.00 (ref.)
	GA	35 (45.45)	139 (55.38)				1.31 (0.77-2.23)
	AA	7 (09.09)	106 (42.23)				4.63(1.46-14.66)
			6 (02.39)				

*: Polymorphic allele; P+: p value corrected for 1,000 permutations

Table 5. Allelic and genotypic frequencies for vesicoureteral reflux (VUR).

		Frequencies					
SNP LIF	**All.**	**VUR N (%)**	**Controles N (%)**	***χ² (df)***	***P***	***P+***	***OR (95%CI)***
rs929271	T	91(70.00)	366 (71.76)	0.16(1)	0.6920	NA	1.00 (ref.)
	G*	39(30.00)	144 (28.24)				1.09(0.71-1.66)
	TT	34(52.31)	134 (52.55)	0.67 (2)	0.7139	NA	1.00 (ref.)
	TG	23(35.38)	98 (38.43)				0.92(0.51-1.67)
	GG	8 (12.31)	23 (09.02)				1.37(0.56-3.33)
rs737812	C	88(68.75)	367 (73.40)	1.08 (1)	1.2980	NA	1.00 (ref.)
	A*	40(31.25)	133 (26.60)				1.25 (0.82-1.91)
	CC	31(48.44)	134(53.60)	1.32 (2)	0.5170	NA	1.00 (ref.)
	CA	26(40.62)	99 (39.60)				1.13 (0.63-2.03)
	AA	7 (10.94)	17 (6.80)				1.78 (0.68-4.66)
rs737921	G	72(56.25)	386 (75.39)	17.37	**3.0711**	**0.010**	1.00 (ref.)
	A*	56(43.75)	126(24.61)	(1)	**1e-005**		2.38 (1.59-3.56)
	GG	16(25.00)	139 (54.30)			**0.010**	1.00 (ref.)
	GA	40(62.50)	108 (42.19)	21.07	**2.6588**		3.22 (1.71-6.05)
	AA	8 (12.50)	9 (3.52)	(2)	**3e-005**		7.72(2.61-22.83)

*: Polymorphic allele; P+: p value corrected for 1,000 permutations

Table 6. Allelic and genotypic frequencies for ureteropelvic junction obstruction (UJO)

		Frequencies					
SNP LIF	**All.**	**UJO N (%)**	**Controls N (%)**	***χ²*** ***(df)***	***p***	***P+***	***OR (95%CI)***
rs929271	T	75 (69.44)	363 (71.74)	0.23	0.6338	NA	1.00 (ref.)
	G*	33 (30.56)	143 (28.26)	(1)			1.12(0.71-1.76)
	TT	25 (46.30)	133 (52.57)		0.5542	NA	1.00 (ref.)
	TG	25 (46.30)	97 (38.34)	1.18			1.37 (0.74-2.53)
	GG	4 (07.40)	23 (09.09)	(2)			0.92 (0.29-2.90)
rs737812	C	87 (80.56)	367 (73.99)	2.14	0.1438	NA	1.00 (ref.)
	A*	21 (19.44)	129 (20.01)	(1)			0.69 (0.41-1.15)
	CC	34 (62.96)	134 (54.03)		0.2531	NA	1.00 (ref.)
	CA	19 (35.19)	99 (39.92)	2.74			0.75 (0.40-1.40)
	AA	1 (01.85)	15 (06.04)	(2)			0.26 (0.03-2.06)
rs737921	G	67 (62.04)	386 (75.98)	8.40	0.0037	0.0080	1.00 (ref.)
	A*	41 (37.96)	122 (24.02)	(1)			1.94 (1.25-3.00)
	GG	20 (37.04)	139 (54.72)		**0.0038**	**0.0140**	1.00 (ref.)
	GA	27 (50.00)	108 (42.52)	11.1			1.73 (0.92-3.26)
	AA	7 (12.96)	7 (02.75)	5 (2)			6.95 (2.20-21.9)

*: Polymorphic allele; P+: p value corrected for 1,000 permutations

Table 7. Allelic and genotypic frequencies for multicystic kidney dysplasia (MKD).

		Frequencies					
SNP LIF	**All.**	**MKD N (%)**	**Controls N (%)**	χ^2 *(df)*	***p***	***P+***	***OR (95%CI)***
rs929271	T	71 (75.53)	366(72.05)	0.49	0.4823	NA	1.00 (ref.)
	G*	23 (24.47)	142 (27.95)	(1)			0.83(0.50-1.39)
	TT	27 (57.45)	134 (52.76)		0.7838	NA	1.00 (ref.)
	TG	17 (36.17)	98 (38.58)	0.48			0.86 (0.44-1.66)
	GG	3 (06.38)	22 (08.66)	(2)			0.67 (0.18-2.42)
rs737812	C	67 (71.28)	366 (73.49)	0.19	0.6582	NA	1.00 (ref.)
	A*	27 (28.72)	132 (26.51)	(1)			1.12 (0.68-1.88)
	CC	22 (46.81)	133 (53.41)		0.5026	NA	1.00 (ref.)
	CA	23 (48.94)	100 (40.16)	1.37			1.39 (0.73-2.63)
	AA	2 (04.25)	16 (06.42)	(2)			0.75 (0.16-3.51)
rs737921	G	59 (65.56)	386 (75.69)	3.89	0.0484	0.10	1.00 (ref.)
	A*	31 (34.44)	124 (24.31)	(1)		59	1.64 (1.01-2.64)
	GG	17 (37.78)	139 (54.51)		0.0933		1.00 (ref.)
	GA	25 (55.56)	108 (42.35)	4.74		NA	1.89 (0.97-3.68)
	AA	3 (06.66)	8 (03.13)	(2)			3.06 (0.74 -12.6)

*: Polymorphic allele; P+: p value corrected for 1,000 permutations

Table 8. Allelic and genotypic frequencies for posterior urethral valves (PUV)

		Frequencies					
SNP LIF	**All.**	**PUV N (%)**	**Controls N (%)**	***χ² (df)***	***p***	***P+***	***OR (95%CI)***
rs929271	T	24 (66.67)	357(71.69)	0.40	0.5258	NA	1.00 (ref.)
	G*	12 (33.33)	141 (28.31)	(1)			1.27(0.62-2.60)
	TT	9 (50.00)	130 (52.21)		0.5869	NA	1.00 (ref.)
	TG	6 (33.33)	97 (38.96)	1.06			0.89(0.30-2.59)
	GG	3 (16.67)	22 (08.83)	(2)			1.97(0.49-7.85)
rs737812	C	24 (70.59)	360 (73.77)	0.16	0.6873	NA	1.00 (ref.)
	A*	10 (29.41)	128 (26.23)	(1)			1.17(0.54-2.52)
	CC	9 (52.94)	131 (53.69)		0.6940	NA	1.00 (ref.)
	CA	6 (35.29)	98 (40.16)	0.73			0.89(0.30-2.58)
	AA	2 (11.76)	15 (06.14)	(2)			1.94 (0.38-9.83)
rs737921	G	17 (47.22)	382 (76.40)	13.1	**0.0002**	**0.0020**	1.00 (ref.)
	A*	19 (52.78)		0(1)	**9**		3.62 (1.82-7.19)
			118(23.60)				
	GG	4 (22.22)				**0.001**	1.00 (ref.)
	GA	9 (50.00)	138 (55.20)	17.2	**0.0001**		2.92 (0.87-9.77)
	AA	5 (27.78)	106 (42.40)	4(2)	**8**		28.75 (6.11 -135.2)
			6 (02.40)				

*: Polymorphic allele; P+: p value corrected for 1,000 permutations

In assessing LIF association with CAKUT, there was no statistically significant evidence for rs 737812 and rs 929271. However, the rs 737921 was associated with both the general CAKUT as with the various phenotypes studied, except for Multicystic Dysplasic Kidney and Hydronephrosis which lose its association after 1,000 permutations. The other phenotypes kept their association even after the test of the 1000 permutations.

In order to check for linkage disequilibrium (LD), HAPLOVIEW was used to calculate the quantities D' which measures the LD itself and r^2 which measures the correlation of the two given SNPs for the three pairs of LIF SNPs. See Table 9. It is concluded the hypothesis of independence of rs 737921 from the two others SNPs studied is reasonably accepted as D' which varies in the range [0 - independent, 1 - dependent] is less than 0.5 and the maximum r^2 is less than 16%.

Table 9. LD analyses.

Linked *loci*	D'	r^2
rs929271 e rs737812	0.882	0.110
rs929271 e rs737921	0.364	0.024
rs737812 e rs737921	0.451	0.158

DISCUSSION AND CONCLUSIONS

Scientists agree that the development of CAKUT probably involves complex interactions among genetic background, mutations, environmental, and epigenetic factors. The aim of this study was to perform a screening for the association of LIF gene polymorphisms with the development of any phenotype of CAKUT or with specific phenotypes in a large sample of patients. Therefore, one has checked for associations with three SNPs covering the entire LIF gene.

In general, the samples analyzed have shown to be in accordance with the principles of Hardy-Weinberg equilibrium - HWE. The investigation routine obeyed all technical rules that might lead to a HWE deviation. In particular, a control of quality of random play of 10% of genotyping as well as negative control in all boards was made without error evidence. Nevertheless, analysis of allele frequencies at HaploView version 4.3 resulted in a slight deviation from HWE (see Table 2) in the case of rs 737921 with a p-value of 0.0424 when it should be above 0.05. An investigation was made to know if the cases were responsible for this disequilibrium. Test of hypothesis using the Chi-Square Distribution concluded that the cases where in HWE with a p value of 0.1585 and the disequilibrium in the controls was confirmed with a p value of 0.0424. Thus, one decided not abandon the results for rs 737921. Justifying this decision, one may refer to Balding (2006) who opines that HWE deviations found only in control groups should be rigorously considered at a significance level $\alpha = 10^{-3}$ or 10^{-4}.

HWE as a strong condition to this kind of investigation has been questioned (CHEN; GAN, 2012; SJAKSE, 2014) with basis on the following fact: ethnic, geographic patterns and specific genetic variations may reflect particular historical processes and adaptation of a population and influence on the current morbidity found on it. The sample was collected on a big metropolitan area of 6 million people. As the disequilibrium is found on the controls, further investigation would be needed to access possible dissent with HWE hypothesis.

Some initial differences between patients and controls were found for SNPs rs 929271 and rs 737812, but they didn't remain after the 1,000 permutations test. Group of patients with UPJO, VUR and PUV presented significant differences on frequencies of the monozygotic ancestral alleles at the marker rs 737921 when compared to controls. The same was not observed in patients with Hydronephrosis or MCDK. An explanation of this result is not found in the literature showing that investigations of the role of LIF gene in human CAKUT must be improved. Some known facts justify research in this direction. Defective expression of LIF can lead to errors in tubulogenesis and in WNT-4 expression. Thus, defective expression of LIF may be responsible for disturbing the role of WNT-4 as transcriptional and signaling gene for nephron tubular development (GLASSBERG, 2002).

On the other side, studies have demonstrated that LIF-pathway has a major role on self-renewal and maintenance of pluripotency of cells. Experiments with mouse embryonic stem cells revealed that LIF acts as an important regulator the self-renewal process (BARASCH, 1999). In vitro research showed that when FGF-2 was placed in a culture medium of mesenchymal cells, it induced mesenchymal cells to cluster but did not induce mesenchymal epithelial conversion. However, when LIF and other cytokines obtained from ureteral bud tissue were added to the culture medium with FGF-2, mesenchymal clustering occurred, followed by conversion into epithelial cells and early tubule formation (GRAF; CASANOVA; CINELLI, 2011). The clinical importance of these findings strengthens the research on LIF´s association to CAKUT.

In this study a statistic association between LIF gene polymorphisms and general CAKUT, UPJO, VUR and PUV was found. Hydronephrosis and MCDK are not found statistically associated with LIF in this study. In more general terms, the study contributes to conjecture that there are specific genetic loci or genetic interactions those

results in specific phenotypes of CAKUT. If it is confirmed by future research, it is a hope that CAKUT phenotypes may be early predicted and treated.

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