Association between the IGF family members and UTUC: a Mendelian randomization study

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The insulin-like growth factor (IGF) system typically includes two ligands (IGF-1 and IGF-2), two receptors (IGF-1R and IGF-2R), seven high-affinity IGF-binding proteins (IGFBPs 1-7), a large group of IGFBP proteases, and novel proteins called low-affinity IGFBP-associated proteins (IGFBP-rPs) [1]. The IGF system is implicated in many diseases, including tumors. And the IGFBP is an important regulator of IGF action, availability, and tissue distribution [2].

Dysregulation of the IGF system is thought to be involved in the development and progression of many tumors. Studies have demonstrated that higher serum IGF-1 levels in the normal range increase the risk of prostate, breast, and colorectal cancers [3]. In a prospective study and Mendelian analysis, higher levels of IGF-2 and IGFBP-3 were found to increase the risk of prostate cancer [4]. In addition, IGF-2 is overexpressed in the urine of bladder cancer patients and may be a biomarker of poor prognosis in transitional cell carcinoma [5].

Upper urinary tract urothelial carcinoma (UTUC) mainly includes renal pelvis cancer and ureteral/urothelial cancer. Although UTUCs have many similarities to bladder cancer, they are different from bladder cancer in clinical and biological characteristics and patient prognosis. As the incidence of UTUC is relatively low, there are few reports on its pathogenesis. Nevertheless, the relationship between the IGF system and UTUC has been reported, though related studies are still scarce and not comprehensive.

It is well known that the occurrence of UTUC is largely related to genetics. In the present study, we used the Mendelian randomization (MR) method to investigate the causal relationship between genetically predicted IGF family members and UTUC.

Methods. ***Study design.*** In this study, serum concentrations of 14 IGF family members (IGF-1, IGF-2R, IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-5, IGFBP-6, IGFBP-7, IGF-1 sR, IGFLR1, CTGF, WISP-1, and CYR 61) were used as exposure factors as reported in previous MR studies [6], and renal pelvis and ureteral/urothelial cancers as outcome variables. The causal relationship between the IGF family members and UTUC was analyzed using a two-sample MR method.

Data sources. Data of genomewide association studies (GWAS) on single nucleotide polymorphisms (SNPs) were derived from publicly available GWAS databases to predict serum levels of the 14 IGF family members using 11036163 associated SNPs in a cohort of 4301 individuals

of European ancestry. Data for renal pelvis and ureteral/urothelial cancers were obtained from the FinnGen R11 database and included 345,287 and 345,184 Finnish individuals, respectively. Additional data are listed in Table I.

MR analysis. First, we established uniform filtering criteria ($p < 5 \times 10^{-8}$) for the instrumental variables (IVs) to ensure statistical significance [7]. However, insufficient SNPs were identified. We then screened SNPs which were strongly correlated ($p < 1 \times 10^{-5}$) with the 14 IGF family members. To avoid bias due to linkage disequilibrium, we performed clumping with $R^2 = 0.001$, kb = 10,000. We then performed a two-sample MR analysis using the two-sample MR package in R (version 4.3.1) [8]. The inverse variance weighted (IVW) method was mainly used in the analysis [9]. In addition, we applied two different MR methods (MR Egger and weighted median (WM)) for supplementary analysis. The MR-Egger regression and the Cochran's Q test were used to determine the presence of pleiotropy and heterogeneity [10].

Statistical analysis. We used the two-sample MR package in R (version 4.3.1) for the statistical analysis. A $p < 0.05$ was considered statistically significant.

Results. After removing linkage disequilibrium, multiple SNPs were found highly associated with the 14 IGF family members, with F statistics > 10 for all instrumental variables. Details of the included SNPs are shown in Supplementary Table SI. In the subsequent MR analysis using the IVW method, IGFBP-6 was found to increase the risk of renal pelvis cancer (odds ratio (OR) = 1.914; 95% confidence interval (CI): 1.031–3.555, $p = 0.040$). It was also found that IGFBP-6 increased the risk of ureteral/urothelial cancer (OR = 2.868; 95% CI: 1.059–7.767, $p = 0.038$), while connective tissue growth factor (CTGF) reduced the risk of ureteral/urothelial cancer (OR = 0.475; 95% CI: 0.243–0.928, $p = 0.029$). However, no causal association with UTUC was found for other IGF family members. The details are shown in Supplementary Table SII. No potential heterogeneity or pleiotropy was found in the sensitivity analysis, as shown in Table II. The scatter plot shows the causal relationship between IGFBP-6 and renal pelvis cancer and ureteral/urothelial carcinoma, and between CTGF and ureteral/urothelial carcinoma (Figure 1). The "leave-one-out" plot suggested that SNPs had no significant impact on the estimated causal association.

Discussion. Urothelial carcinoma is one of the most common malignant tumors of the urinary system, which is divided into renal pelvis and ureter/urothelial carcinoma (commonly referred to as UTUC) and bladder urothelial carcinoma. The incidence of UTUC is lower than that of bladder urothelial carcinoma, and its exact cause remains unclear. In this study, we investigated the causal relationship between the IGF family members and UTUC by the MR method and identified that higher IGFBP-6 serology increased the risk of renal pelvis and ureteral/urothelial cancers, while a lower CTGF level increased the risk of ureteral/urothelial cancer.

There have been few reports on the relationship between the IGF family members and UTUC. Eich *et al*. [11] found that IGF1R was overexpressed in 70% of UTUC cases. Liang *et al*. [12] showed that the expression of IGFBP-5 was related with the

Table I. The details of the exposures and outcomes

Phenotype	GWAS ID	Sample size	Number of SNPs	Population
IGF-1	prot-c-2952_75_2	1000	501,428	European
IGF-2R	prot-c-3676_15_3	1000	501,428	European
IGFBP-1	prot-c-2771_35_2	1000	501,428	European
IGFBP-2	prot-c-2570_72_5	1000	501,428	European
IGFBP-3	prot-c-2571_12_3	1000	501,428	European
IGFBP-4	prot-c-2950_57_2	1000	501,428	European
IGFBP-5	prot-c-2685_21_2	1000	501,428	European
IGFBP-6	prot-c-2686_67_2	1000	501,428	European
IGFBP-7	prot-c-3320_49_2	1000	501,428	European
IGF-1 sR	prot-c-4232_19_2	1000	501,428	European
IGFLR1	prot-a-1455	3301	10,534,735	European
CTGF	prot-c-2975_19_2	1000	501,428	European
WISP-1	prot-c-3057_55_1	1000	501,428	European
CYR61	prot-a-758	3301	10,534,735	European
Renal pelvis carcinoma	finn-b-C3_RENAL_PELVIS_EXALLC	345287	21,304,491	European
Ureteral/urothelial carcinoma	finn-b-C3_URETER_EXALLC	345184	21,304,446	European

Table II. Pleiotropy and heterogeneity test of IGF family members in GWAS for renal pelvis and ureteral/urothelial carcinoma

Exposure	Outcome	Heterogeneity test			Pleiotropy test		
		Q	Q_df	*P*-value	Intercept	SE	*P*-value
IGF-1	Renal pelvis Ca	0.537	5	0.991	0.014	0.218	0.952
	Ureteral/urothelial Ca	2.186	5	0.823	0.266	0.339	0.477
IGF-2R	Renal pelvis Ca	0.277	4	0.991	–0.036	0.117	0.780
	Ureteral/urothelial Ca	2.519	4	0.641	–0.004	0.186	0.985
IGFBP-1	Renal pelvis Ca	5.830	3	0.120	–0.570	0.266	0.165
	Ureteral/urothelial Ca	3.527	3	0.317	–0.183	0.551	0.771
IGFBP-2	Renal pelvis Ca	3.940	3	0.268	–0.269	0.312	0.480
	Ureteral/urothelial Ca	2.623	3	0.453	–0.015	0.465	0.977
IGFBP-3	Renal pelvis Ca	10.981	7	0.139	–0.162	0.232	0.512
	Ureteral/urothelial Ca	7.666	7	0.363	0.495	0.284	0.132
IGFBP-4	Renal pelvis Ca	0.242	1	0.623	NA	NA	NA
	Ureteral/urothelial Ca	0.134	1	0.714	NA	NA	NA
IGFBP-5	Renal pelvis Ca	3.580	3	0.311	–0.167	0.278	0.608
	Ureteral/urothelial Ca	0.488	3	0.922	0.205	0.363	0.629
IGFBP-6	Renal pelvis Ca	0.204	2	0.903	–0.041	0.243	0.893
	Ureteral/urothelial Ca	1.554	2	0.460	–0.074	0.481	0.903
IGFBP-7	Renal pelvis Ca	0.430	2	0.806	0.069	0.278	0.845
	Ureteral/urothelial Ca	2.670	2	0.263	0.421	0.577	0.598
IGF-1 sR	Renal pelvis Ca	1.081	3	0.782	–0.043	0.233	0.871
	Ureteral/urothelial Ca	8.559	3	0.036	–0.497	0.700	0.552
IGFLR1	Renal pelvis Ca	26.952	28	0.521	–0.079	0.064	0.227
	Ureteral/urothelial Ca	20.815	28	0.833	0.059	0.101	0.565
CTGF	Renal pelvis Ca	5.332	6	0.502	–0.270	0.312	0.427
	Ureteral/urothelial Ca	1.379	6	0.967	0.344	0.494	0.518
WISP-1	Renal pelvis Ca	0.749	2	0.688	0.408	0.475	0.548
	Ureteral/urothelial Ca	5.681	2	0.058	–0.945	1.512	0.645
CYR61	Renal pelvis Ca	30.445	21	0.083	–0.074	0.088	0.410
	Ureteral/urothelial Ca	17.061	21	0.707	0.213	0.115	0.078

IGF – insulin-like growth factor, GWAS – genomewide association study, Ca – carcinoma.

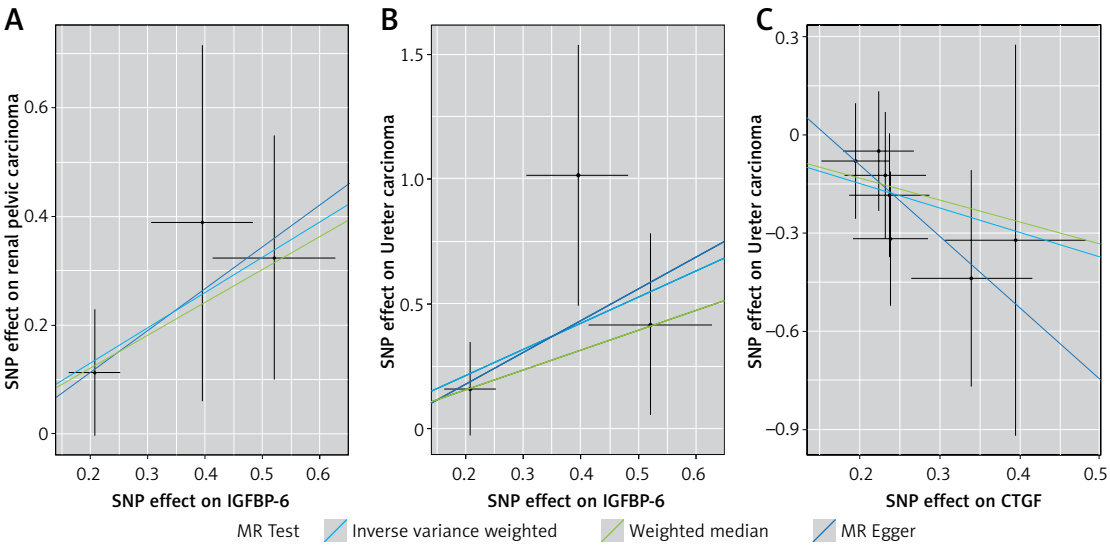

Figure 1. Scatter plot: **A** – The causal relationship between IGFBP-6 and renal pelvis carcinoma. **B** – The causal relationship between IGFBP-6 and ureteral/urothelial carcinoma. **C** – The causal relationship between CTGF and ureteral/urothelial carcinoma

clinicopathological features of UTUC and bladder urothelial carcinoma, while its overexpression was associated with poor clinical prognosis.

The effect of the IGF is regulated by specific high-affinity binding proteins, where IGFBP-6, different from IGFBP-1-5, has a 50-fold higher binding preference for IGF-2 than for IGF-1 [13], so IGFBP-6 might be a specific inhibitor of IGF2 action. Many previous studies have demonstrated that IGFBP-6 level was decreased in cancer, while some studies revealed that IGFBP-6 and other IGF system members were upregulated in the process of prostate epithelial cell differentiation [14]. In transformed human prostate epithelial cells, which are more tumorigenic, the expression of IGFBP-6 and other IGFBPs was decreased [15]. In addition, IGFBP-6 expression was decreased in rhabdomyosarcoma (RMS), lung cancer, breast cancer, colon cancer and other cancers. However, IGFBP-6 expression was increased in pancreatic cancer and adrenocortical carcinoma [16]. Our study also found an association of the increased IGFBP-6 serum level with the risk of renal pelvis and ureteral/urothelial cancers. The increased expression of IGFBP-6 might be a compensatory response to increased IGF-2 activity or reflect an IGF-independent action of IGFBP-6.

CTGF is composed of an N-terminal secretory signal peptide (S) and four modular domains including IGFBP [17], forming the IGFBP superfamily with IGFBP-1-6. The expression of CTGF decreases in many cancers, including Wilms tumor and medulloblastoma, and could be used to predict the prognosis of cancer patients [17]. Our MR study also showed that decreased levels of CTGF increased the risk of ureteral/urothelial cancer, which was not reported in previous studies and may provide new insights for/into the screening of tumor markers of ureteral/urothelial cancer.

Our study has several advantages. Firstly, the study design of MR effectively reduces the confounding factor interference, and we conducted a sensitivity analysis to make the results more reliable. Secondly, we utilized data from publicly available GWAS summary surveys, benefiting from a large sample size, which enhances the robustness of our analysis.

However, there are some limitations in this study. We adopted a $p < 1 \times 10^{-5}$ threshold when selecting a strongly associated SNP from the GWAS dataset of the IGF family members, since it is impossible to obtain enough SNPs using a $p < 5 \times 10^{-8}$ threshold for MR analysis. The precise function of the selected SNPs remains unclear. These genetic variants have not been identified as biological links to exposure to the IGF family members, suggesting a potential bias in causal estimates.

In conclusion, our MR study has provided strong evidence that high levels of IGFBP-6 in the IGF family members are associated with the increased risk of renal pelvis and ureteral/urothelial cancers, while low levels of CTGF are associated with the increased risk of ureteral/urothelial cancer. These findings provided new insights into the screening of potential specific tumor markers of UTUC and the subsequent targeted therapy. Definitely, this still needs to be confirmed by more thorough molecular and mechanistic studies.

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Ethical approval

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

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