A GENOMIC ANALYSIS OF MONOZYGOTIC TWINS DISCORDANT FOR THE EXPRESSION OF MEGAURETER USING THE WHOLE EXOME TECHNIQUE

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INTRODUCTION

Congenital anomalies of the kidney and urinary tract (CAKUT) represent a broad range of disorders that result from abnormalities of the urinary collecting system, abnormal embryonic migration of the kidneys or abnormal renal parenchyma development. These disorders are commonly found in humans, accounting for 20 to 30% of all malformations diagnosed during the prenatal period. Megaureter constitutes one of the phenotypes of CAKUT and represents a condition whereby the ureter is abnormally dilated. Historically, our knowledge about the pathogenesis of human diseases has greatly increased because of studies with monozygotic twins. Theses studies can potentially help to distinguish the relative contributions of genetic and environmental factors in the pathogenesis of human diseases.

FIGURE 1 – THE URINARY TRACK EARLY ORGANOGENESYS

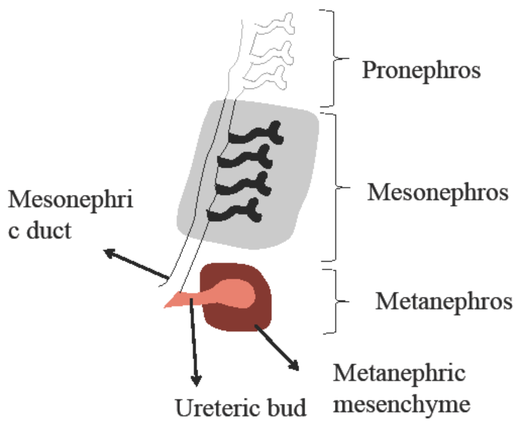


TABLE 1: MAIN SINGLE-GENE MUTATIONS ASSOCIATED WITH CAKUT

GENE	PHENOTYPE
AGTR2	UPJ obstruction, megaureter, MCDK, hydronephrosis, PUV
BMP4	Renal hypodysplasia
EYA1	Branchio-oto-renal (BOR) syndrome
PAX2	Hipoplasia renal, coloboma renal
SALL	Townes-Brocks Syndrome
SIX1	Branchio-oto-renal (BOR) syndrome
SIX5	Branchio-oto-renal (BOR) syndrome

UPJ: ureteropelvic junction
MCDK: multicystic dysplastic kidney
PUV: post-uretral valve

OBJECTIVES

This study aimed to investigate a pair of monozygotic twins discordant for megaureter and his parents for genetic variants using the Whole Exome technique.

PATIENTS AND METHODS

This study included 11 non-related individuals with defined diagnosis of primary megaureter and a pair of monozygotic twins discordant for megaureter followed at the Pediatric Nephrology Unit of the Hospital Bias Fortes - Universidade Federal of Minas Gerais (UFMG). All individuals were prenatally screened to detect primary megaureter. The screening protocol consisted in a detailed ultrasound (US) scan performed after 28 weeks of gestation at the Fetal Medicine Division from our institution. Megaureter in fetal US was considered to be present if the ureter diameter were superior to 7 mm. All infants underwent systematic postnatal investigation and were prospectively followed up at Pediatric Nephrology Unit.

The local Ethics Committee evaluated and approved this study in accordance with the principles outlined in the Declaration of Helsinki. Informed consent was obtained from all participants. After informed consent, participants underwent an intravenous puncture to collect 15 ml of peripheral blood samples. DNA was extracted from peripheral blood lymphocytes in accordance with the method described by Lahiri and Nurnberger (1991).

The extracted DNA was then sequenced using the Whole Exome technique. The monozygotic twins and his parents had their DNA sequenced separately. The DNA of the other participants were pooled to optimize the results. The main following steps were taken to prioritize the high quality variants: (i) variants within intergenic, intronic, and UTR regions and synonymous mutations were excluded; (ii) variants with quality score minor than 20 were excluded; (iii) only the conservation score (phyloP) higher than 3 were consider; (iv) After this prior selection, the remained genes were filtered by the function. We used the Grantham score to estimate the mutation pathogenicity to the resulting protein and the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) to filter and assess the results. The final set of selected variants was visually inspected using Integrative Genomics Viewer (IGV ver 2.3.40) .

FIGURE 2 – HEREDOGRAM FROM THE FAMILY UNDER STUDY

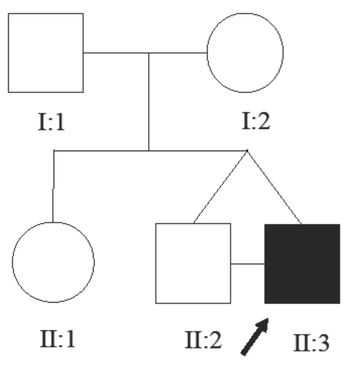
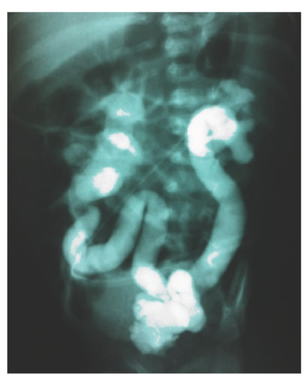


FIGURE 3 –THE AFFECTED TWIN PHENOTYPE

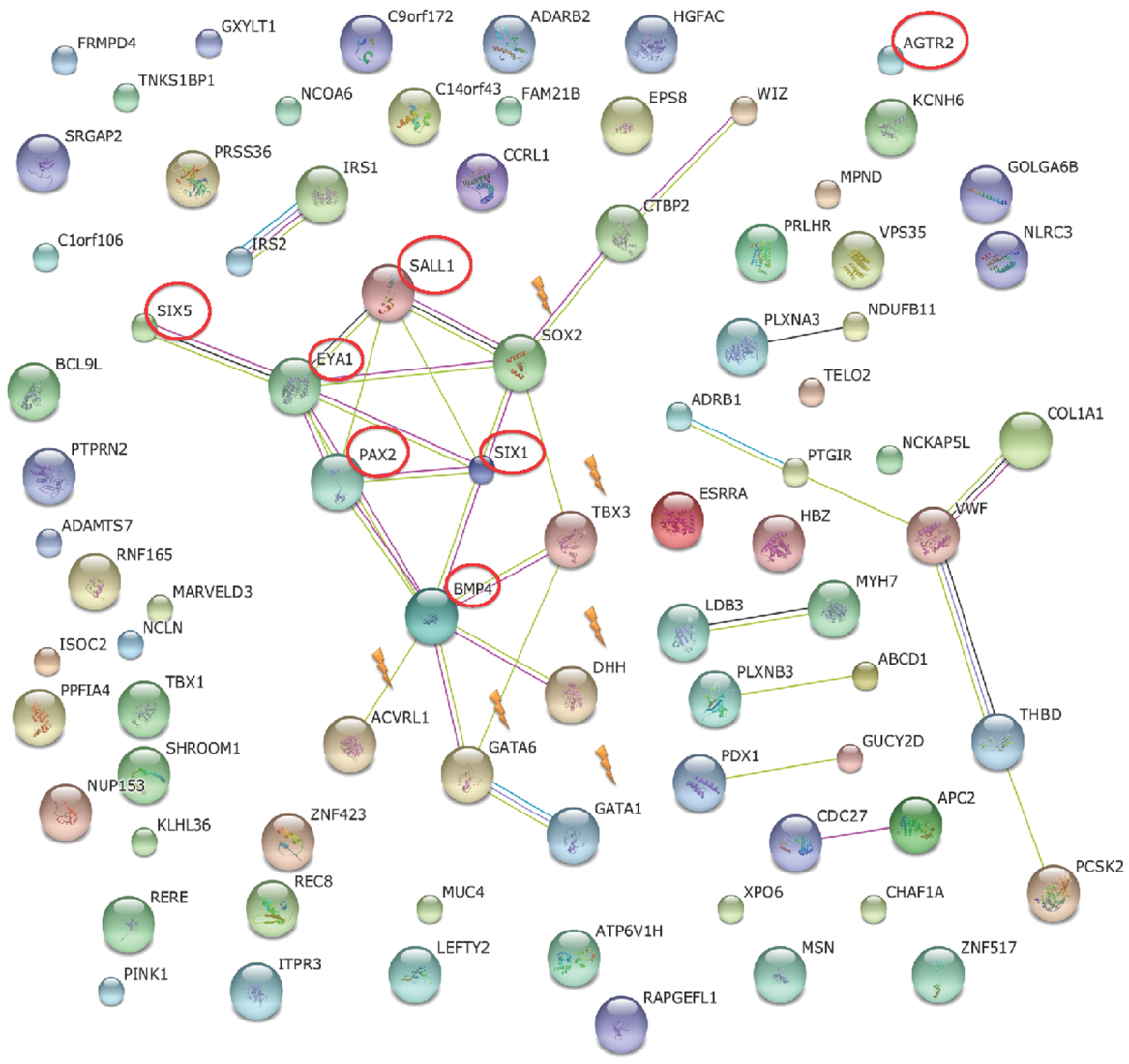


RESULTS

We identified differential single nucleotide variations present exclusively in the proband in the following genes (n=81; decreasing Grantham score order): C9orf172; HGFAC; RAPGEFL1; ACVRL1; THBD; NCKAP5L; PCSK2; VWF; ADRB1; XPO6; ADARB2; FAM21B; GATA6; LEFTY2; NDUFB11; TBX3; PPFIA4; RNF165; MAGEL2; REC8; GATA1; CHAF1A; PTGIR; ADAMTS7; KLHL36; MPND; SOX2; GUCY2D; GXYLT1; MSN; PDX1; SRGAP2; ABCD1; ISOC2; WIZ; NCLN; MUC4; C1orf106; APC2; IRS2; ISOC2; LDB3; SHROOM1; CDC27; DHH; RERE; ZNF517; CCRL1; IRS1; TNKS1BP1; BCL9L; MAGEL2; MSN; PTPRN2; HBZ; CDC27; MARVELD3; TBX1; ZNF423; KCNH6; PINK1; COL1A1; FRMPD4; GOLGA6B; PLXNB3; ITPR3; PLXNA3; PRLHR; LRRC73; ESRRA; PRSS36; C14orf43; NLRC3; TELO2; CTBP2; NUP153; ATP6V1H; EPS8; MYH7; VPS35; NCOA6.

When we compared the exclusive variations found in the proband with the variations found in the pool of megaureter non-related patients, there were 4 variations in common: REC8; LDB3; NUP153; NCOA6; VPS35. Previously described polymorphic variants in public data were investigated and compared with the variations found in the current Exome. These variants were not found in previous exomes (http://evs.gs.washington.edu/EVS/).

FIGURE 4 – THE SEARCH TOOL FOR THE RETRIEVAL OF INTERACTING GENES/PROTEINS (STRING) ANALYSIS



CONCLUSION

Gene-targeting experiments have greatly improved our understanding of kidney and urinary tract morphogenesis leading to several genes that are believed to play a role in the normal development of the kidney and the urinary tract. This study aimed to contribute to the investigation of the molecular basis of the primary megaureter in the Brazilian population. With this study new genes have emerged as possible candidates in the development of the primary megaureter, with special interest in the TBX3, GATA6, GATA1, DHH, ACVRL1 and SOX2. Our next step is to use the Polymerase chain reaction (PCR) Sanger sequencing method to confirm our findings and test the presence of these variations in a larger sample.

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