*Helen M. Stuart, BMed Sci, MB ChB, Msc and Adrian S. Woolf, MA, MD**

Genetics of Renal Tract Malformations

**Institute of Human Development, Faculty of Medical and Human Sciences, University of Manchester, Manchester Academic Health Science Centre (MAHSC) and the Royal Manchester Children's and St Mary's Hospitals, Manchester M13 9WL, UK*

Introduction

Renal tract malformations (RTMs) (*i.e.* those affecting the kidneys and/or lower urinary tract) represent deviations from normal development. They are common, for example being diagnosed by antenatal ultrasonography in 0.16% births, representing one tenth of all anomalies diagnosed in this manner.[1] Normal development is a carefully choreographed process, the instructions for which are contained in an individual's genes. Humans have approximately 30,000 pairs of genes packaged on 23 pairs of chromosomes (22 autosomes and one sex chromosome pair); additionally, each person has 37 genes within mitochondria. When a gene is 'expressed' it provides instructions to produce a particular protein or, in some cases, a functional RNA molecule. Renal tract development is characterised by changing patterns of expression of many hundreds of genes, from the inception of the kidney,[2] ureter[3] and bladder,[4] through the growth of these rudiments into functional, mature organs. Indeed, in the last two decades it has been established that human RTMs can be caused by mutation of many genes.[5, 6] Such knowledge is beginning to impact on clinical practice, giving families of affected individuals reasons why these sometimes clinically-devastating anomalies arose.[5] Many of the genes essential for renal tract development also operate in other organ systems, proving one explanation why one third of RTMs are 'syndromic', occurring in association with specific patterns of extra-renal malformations.[1, 6] In this chapter, to introduce readers to this relatively new field of the genetics of human RTMs, we discuss some general genetic concepts as well as mentioning specific diseases.

Mendelian Inheritance

Some mutations are inherited from one or both of an individual's parents, explaining why genetic diseases can recur in any one family (Fig. 1). This is called 'Mendelian inheritance' after Gregor Mendel, the 19th century Austrian monk who studied inheritance patterns of pea plant characteristics. In routine clinical practice, many RTMs are seemingly sporadic or affect more than one individual within a family in a complex (non-Mendelian) pattern, making it difficult to discern a precise genetic contribution. Having said this, some RTMs are inherited in a Mendelian manner and Table 1 lists a selection of them. Familial genetic 'linkage' analysis is a powerful technique for identifying genomic regions associated with a condition in affected family members. It has led to the discovery of many RTM genes and continues to be an important technique in genetic research. For some syndromes featuring RTMs, mutations in more than one gene can cause an identical clinical disease or 'phenotype'. Such syndromes include Alagille, branchio-oto-renal and Fraser syndromes (Table 1). In these cases, the gene products either have a related function or form critical components of a common biological pathway operating during organogenesis.

Autosomal Recessive Disease

A disease is 'autosomal recessive' (AR) when mutations in both copies, or 'alleles', of a gene (*i.e.* one on each of a chromosome pair) are required to cause disease (Fig. 1, left-hand panels). Some AR disorders associated with RTMs are listed in Table 1. These mutations are usually inherited from carrier, or 'heterozygous', parents who have one normal copy and one mutated copy of the gene, with a 1 in 4 risk of recurrence of the disease in a future child. AR mutations usually cause a 'loss of function' of the gene such that an affected individual has an absent or very low level of the molecule normally encoded by the gene. Carriers are usually well because having just one functional gene copy is sufficient to maintain healthy cells. Although the genome of each 'normal' human carries numerous loss-of-function mutations in different genes, each AR disease is rare because of the low probability that two individuals, each carrying a mutation for a particular gene, will meet and procreate. On the other hand, AR diseases are commoner in consanguineous relationships where both parents may carry a mutation inherited from a common ancestor.

During the initiation of the embryonic metanephric kidney, the ureteric bud, a branch from the distal end of the Wolffian (mesonephric) duct, grows into adjacent intermediate mesoderm and these two tissues interact, respectively ultimately forming ureteric/collecting duct epithelia and kidney nephrons. The FRAS gene family code for basement membrane proteins which coat the external surface of the ureteric bud and they are required

for tissue inductions.[7] Mutations of these genes lead to renal and ureteric agenesis in the often fatal Fraser syndrome, an AR disorder which also features hidden eyes ('cryptophthalmos'), soft tissue fusion of fingers and toes ('syndactyly'), laryngeal anomalies and ambiguous genitalia.[8] Of note, the addition of nephrogenic growth factors rescues renal agenesis in an animal model of the human syndrome.[7]

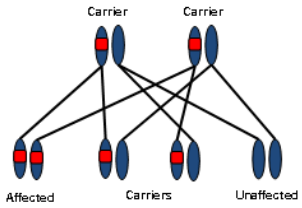
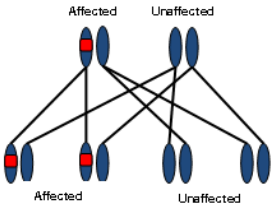
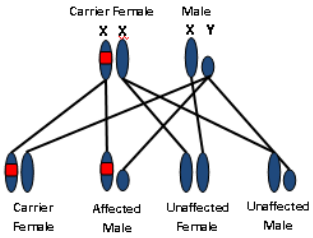
Mode of Inheritance	Autosomal Recessive	Autosomal Dominant	X-Linked Recessive
Offspring outcomes Chromosome Chromosome with mutated gene			
Is the mutation a result of transmission from parents or can it occur *de novo*?	Usually inherited	Either is possible. The proportion of new mutations increases with increased severity of condition	Either is possible. Often a new mutation has occurred in the mother of an affected male
Penetrance of the mutation	Usually 100% in those carrying two mutant alleles/genes. Rare for carriers (i.e. those with just one mutant allele/gene) to have clinical features	Often less than 100% in those carrying the mutant allele/gene.	Usually 100% in males carrying a mutant allele/gene. Carrier females may have disease with severity depending on pattern of X-inactivation
Variability of expression	Usually less than in dominant disease	Usually significant variability in affected individuals, even in a single kindred	Usually less than in dominant disease

Fig. 1. Summary of characteristics of the three main types of Mendelian inheritance.
Key to Fig. 1. 'Penetrance' refers to the observation that the proportion of those affected by the clinical disorder is less than 100% of expected based on whether individuals carry a the mutation. 'Variability of expression' refers to the observation that affected individuals have a spectrum of disease severity. With respect to RTMs, modifying genes and yet-to-be defined alterations of fetal environment may contribute to the variability in the occurrence and/or severity of the genetic disease.

The uro-facial, or Ochoa, syndrome is a good example of an AR disease which affects the bladder.[9] Here, although the bladder begins to form, affected individuals suffer from congenital bladder dysfunction characterised by failure of complete voiding accompanied by upper tract dilatation. A diagnostic clue, evident in the clinic, is that such individuals have a characteristic grimace when smiling. Some individuals with the syndrome have been found to have mutations of a gene called HPSE2 which codes for heparanase.[2] This molecule is predicted to modify growth factor signalling and/or cell adhesion during development and must somehow facilitate detrusor maturation and/or contraction. The gene is expressed in bladder muscle as well as in the central nervous system.[10] Bladders in the uro-facial syndrome share some biological similarities with those found in the prune belly syndrome e.g. failure to empty and abnormal walls with diverticula. Prune belly syndrome can also be inherited in an AR manner[11] and, it can be caused by mutation of the CHRM3 gene which encodes the M3 acetylcholine receptor which is required for detrusor contraction.

Autosomal Dominant Disease

'Autosomal dominant' (AD) diseases arise when a mutation in only one of a gene pair is sufficient to cause biological dysfunction. Some AD syndromes associated with RTMs are listed in Table 1. AD conditions can be inherited from an affected parent, with a 1 in 2 risk of recurrence in future children (Fig. 1, central panels). It is often observed that, in AD disorders, a parent carrying the mutation may be clinically unaffected or have only a mild version of the disease,[12] situations respectively called 'non-penetrance' and 'variable expressivity'. Both phenomena may be explained by: 1. the modifying actions of poorly-recognised environmental factors affecting the fetus, such as variations in maternal protein and vitamin intake;[13] 2. the presence in the individuals of variants of other genes which affect renal tract development. As an example of the latter, Weber *et al*[14] showed that the severity of RTMs within a family with mutation of the PAX2 (Paired box-2) gene was increased by the presence of a variant in another gene, SIX1. A common feature of AD disease is the discovery that a mutation is apparently new ('de novo') in the affected child, likely having occurred during production of the egg or sperm. Alternatively, a clinically unaffected parent could be a genetic 'mosaic', carrying a de novo mutation which

arose after the start of his or her own development, resulting in colonies of cells either having or lacking the mutation. If such abnormal colonies give rise to the germ cells, the mutation could be recurrently passed on so that all of an offspring's cells carry the genetic lesion.[15]

TABLE 1. Some Defined Genetic Syndromes Featuring RTMs

Syndrome name (Acronym; OMIM number)	RTMs	Extra-renal features	Mutated gene(s) (function of encoded protein)
Autosomal Recessive			
Fanconi anaemia (227650)	EK, CK, HYD, RA, RD, RH, VUR	Bone marrow failure, microcephaly, MR, radius malformation, tumour predisposition	15 genes implicated thus far (DNA repair)
Fraser (219000)	RA, RD	Cryptophthalmos, laryngeal stenosis, Mullerian duct malformation, syndactyly.	*FRAS1*, *FREM1* and *FREM2* (basement membrane proteins)
Smith-Lemli-Opitz (270400)	RD	Ambiguous genitalia, cataracts, cleft palate, CHD, facial dysmorphology, microcephaly, MR, polysyndactyly	*DHCR7* (sterol biosynthesis)
Uro-facial or Ochoa (236730)	Bladder dysmorphology and voiding dysfunction, HYD, VUR	Abnormal expression when smiling, constipation	*HPSE2* (heparanase modifier)
Autosomal dominant			
Alagille (118450)	PUJO, RH, RD, renal tubular acidosis	Bile duct dysmorphogenesis, CHD, skeletal abnormalities.	*JAG1* and *NOTCH2* (signalling pathway)
Branchio-oto-renal (BOR; 113650)	HYD, PUJO, RA, RD, RH, VUR	Branchial fistula and cyst, deafness, dysplastic ear, lacrimal duct agenesis, preauricular pit	*EYA1* and *SIX1* (regulate gene expression)
Coloboma, heart anomaly, choanal atresia, retardation, genital and ear (CHARGE; 214800)	CK, HYD, RA, VUR	CHD, coloboma (various eye malformations), choanal atresia, ear abnormalities, cryptorchidism, deafness, genital abnormalities, tracheoesophageal fistula.	*CHD7* (regulates gene expression)
Hand-foot-genital (140000)	HYD, hypospadias, PUJO, VUR	Hypoplastic thumb/fingertips, Mullerian duct malformation	*HOXA13* (transcription factor)
Hypoparathyroidism, deafness and renal (HDR; 146255)	RD, VUR	Deafness, hypocalcaemia	*GATA3* (transcription factor)
Okihiro (607323)	Bladder diverticula, EK, HK, RA, RD, VUR	CHD, deafness, Duane (cranial nerve VI) anomaly, thumb and arm malformations	*SALL4* (transcription factor)

Pallister-Hall (146510)	HYD, RA, RD, RH, VUR	Bifid epiglottis, hypothalamic hamartoma, imperforate anus, polydactyly.	*GLI3* (transcription factor)
Renal-coloboma (120330)	HYP, RD, RH, VUR	Optic nerve coloboma, deafness	*PAX2* (transcription factor)
Renal cysts and diabetes (RCAD; 137920)	HK, HYD, prune belly syndrome, RD, RH	Diabetes mellitus, gout, hypomagnesaemia, Müllerian duct malformations	*HNF1B* (also called *TCF2*) (transcription factor)
Schinzel Giedion (269150)	HYD, VUR	CHD, MR, seizures, skull dysmorphology, hypertrichosis	*SETBP1* (unknown)
Townes-Brocks (107480)	PK, HK, RD, VUR, ureteral valves	Anal atresia, CHD, deafness, sensaric thumb malformations	*SALL1* (transcription factor)
X-linked			
Kallmann (308700)	RA, RD, VUR	Anosmia, hypogonadotropic hypogonadism	*KAL1* (cell adhesion/ migration)
Otopalatodigital spectrum (311300)	HYD, hypospadias, VUR, Urethral and ureteric obstruction	Cleft palate, deafness, skeletal dysplasia.	*FLNA* (actin-binding cytoskeleton)
Simpson-Golabi-Behmel (312870)	DK, HYD, hypospadias, nephromegaly, RD, renal tumours	CHD, cryptorchidism, diaphragmatic hernia, MR, overgrowth, polydactyly, tumour predisposition	*GPC3* (heparan sulphate proteoglycan)

Within cohorts affected by apparently non-syndromic RTMs, a proportion are caused by AD mutations. In groups of individuals with renal hypoplasia or dysplasia, up to 20% have been found to have mutations in PAX2 or HNF1B (Hepatocyte nuclear factor 1B).[14,16,17] Both genes are prominently expressed in the ureteric bud/collecting duct lineage and code for transcription factors, proteins which bind to DNA and regulate the activity of numerous target genes. A key function of the PAX2 protein is to prevent excess apoptotic death of precursor cells in the metanephros, and thus PAX2 mutations usually cause renal hypoplasia i.e. small kidneys with a severe nephron deficit. The HNF1B protein stimulates epithelia to differentiate and prevents uncontrolled proliferation and cyst formation. Thus the multicystic dysplastic kidney is a common RTM associated with HNF1B mutation. As noted in Table 1, mutations of both genes can result in extra-renal disease (diabetes mellitus with HNF1B mutation and blindness with PAX2 mutation); importantly, however, these features are often absent in the patients presenting to Urologists and Nephrology clinics. The proportion of RTMs attributed to Mendelian mutations is likely to increase dues to wider availability of genetic testing and the continuing discovery of new genes associated with RTMs.

X-linked Inheritance

Conditions caused by mutations in genes on the X chromosome are 'X-linked' and some such syndromes associated with RTMs are listed in Table 1. Females (XX) have two copies of an X-linked gene whilst males (XY) have only one (Figure 1, right-hand panels). This means a male with an X-linked mutation will be affected. Females carrying an X-linked condition have one mutated and one normal copy of the gene. Some X-linked mutations are recessive, only causing disease in males, while others are dominant and affect both males and females who carry one mutated allele. One copy of the X chromosome in each of a female's cells has undergone random inactivation in early embryogenesis, a process called 'lyonization' after the geneticist Mary Lyon who first proposed the phenomenon. This means that females carrying a mutation of a gene on one X chromosome are in fact mosaics for different proportions of cells expressing the normal or the mutated gene. This idea helps to explain why such females may be less, or more, affected by the associated genetic disease.

An example of an X-linked recessive disorder featuring RTMs is provided by the X-linked form of Kallmann syndrome. As in Fraser syndrome, discussed above, the mutated gene normally codes for a protein which coats the surfaces of embryonic kidney epithelial cells. When the protein is absent, renal agenesis is the result, usually manifest as a solitary functioning kidney.[18] Other features of the syndrome are hypogonodotrophic hypogonadism (manifest by failure to proceed through puberty) and anosmia (absent sense of smell) and both are explained by the fact that the gene is also expressed in the forebrain both in nerves generating hypothalamic hormones as well as in olfactory neurones.[19]

TABLE 2. Gross Chromosomal Abnormalities Associated with RTMs

Syndrome name; chromosome affected [OMIM number]	RTMs (collective frequency, if known)	Selected extra-renal features
Cat eye; tetrasomy 22q (115470)	HYD, RA, RD, VUR (unknown)	Anal atresia+/-fistula, CHD, coloboma, (eye malformation), MR
DiGeorge or velocardiofacial; 22q11 deletion (188400)	DK, HK, HYD, RA, RD, VUR (30%)	CHD, cleft palate, facial dysmorphology, hypocalcaemia, immune deficiency, MR
Down, trisomy 21 (190685)	CAKUT, DK, HK, HYD, PUJO, RA, RD, VUR (15%)	CHD, facial dysmorphology, dysmorphic features, hypotonia, gastrointestinal malformations, haematological malignancies, MR
Edward, trisomy 18	CAKUT, DK, HK, RA, RD (70%)	CHD, facial dysmorphology, MR, microcephaly
Miller-Dieker lissencephaly; 17p13 deletion (247200)	DK, RA, RD, VUR (unknown)	CHD, MR, facial dysmorphology, genital hypoplasia
Patau, trisomy 13	CAKUT, DK, HK, RD, (70%)	Cleft lip+/-palate, holoprosencephaly, MR, polydactyly
Turner; monosomy X or X+X rearrangement	DK, HK, PUJO, RD (70%)	CHD, facial dysmorphology, gonadal dysgenesis, short stature
Williams Beuren; 7q11 deletion (194050)	Bladder diverticulae and overactivity, RA, renal artery stenosis, VUR (30%)	CHD, facial dysmorphology, hypercalcaemia, MR, short stature
Wolf-Hirschhorn; 4p deletion (194190)	Bladder exstrophy, HK, RA, RD, VUR (30%)	CHD, deafness, facial dysmorphology, hypotonia, MR, short stature.

"CHD = congenital heart disease; EK = ectopic kidney; HK = hypoplastic kidney; HYD = hydronephrosis; OMIM = Online Mendelian Inheritance in Man; PUJO = pelviureteric junction obstruction; RA = renal agenesis; RD = renal dysplasia; VUR = vesicoureteric reflux; MR = mental retardation "

Chromosomal Abnormalities and RTMs

RTMs occur in around one third of individuals with gross chromosomal abnormalities (deletions, duplications or rearrangements), with some commoner examples listed in Table 2. In each of these cases, the structure and thus function of numerous genes may be compromised. At least for multicystic dysplastic kidneys, such gross genetic lesions are more likely to be found when the RTM is bilateral and when extra-renal anomalies are present.[20] Because the biological effects of gross chromosomal abnormalities can be devastating, they often appear as de novo events, absent in parents of affected individuals. Such new mutations may be generated during the production of the egg or sperm which then goes on to make an individual, or at the very start of embryonic development, so that all cells in the body carry the mutation. Detailed analysis of chromosomal copy number variation (CNV) undetectable by classical cytogenetic techniques is now possible by array comparative genomic hybridisation or simply 'microarray'. Using this technique: CHD7 (Chromodomain-helicase-DNA-binding protein 7) was implicated in CHARGE (coloboma, heart anomaly, choanal atresia, retardation, genital and ear) syndrome;[21] a recurrent microdeletion on the long arm of chromosome 17 including HNF1B was found to be associated with features of RCAD (renal cysts and diabetes) syndrome with neurocognitive disability;[22] and 22q11 duplications were associated with bladder exstrophy.[23]

Genetic Complexity and Human RTMs

Non-syndromic primary VUR is a common condition, affecting at least 0.5-1% of young children. The incidence of reflux is increased in first degree relatives and families are commonly seen with two or more affected siblings.[24] Frustratingly, we lack precise genetic explanations for the great majority of individuals with this condition. Most likely, whether or not an individual is born with primary VUR will be determined by more than one genetic mechanism. For some families, the disorder is Mendelian,[25] and will be caused by mutation of any one of the many genes which drive ureteric growth. As is evident from Table 1, VUR has been reported in many syndromes, suggesting that it can result from mutation of any one of numerous genes active in renal tract development. Indeed, rare individuals with VUR who lack extra-renal anomalies have been reported with

mutations of genes which direct the initiation and differentiation of the ureter.[26,27] Perhaps the proportion of VUR attributed to Mendelian mutations will increase due to the continuing discovery of new genes associated with RTMs combined with 'next generation' genetic technologies which rapidly sequence coding regions from an individual's whole genome. In other individuals, VUR may be the result of the interaction of minor variations, or 'polymorphisms', of several genes coding for molecules which act in common or interacting pathways. Using groups of several hundred of affected individuals, attempts are being made using 'GWAS' (genome-wide association studies) to address this issue but, thus far, results have been disappointing.[28] Probably, much larger collections of patients will be need to be analysed to generate results with the statistical power to implicate specific gene variants, as has been accomplished with other kidney disorders.[29]

Acknowledgements

The authors acknowledge support from the NIHR Manchester Biomedical Research Centre, the Wellcome Trust, Kids Kidney Research and Kidney Research UK.

Reference:

1. Wiesel A, Queisser-Luft A, Clementi M, Bianca S, Stoll C; EUROSCAN Study Group. Prenatal detection of congenital renal malformations by fetal ultrasonographic examination: an analysis of 709,030 births in 12 European countries. *Euro J Med Genet*, 48: 131-144, 2005.
2. Price KL, Long DA, Jina N, Liapis H, Hubank M, Woolf AS, Winyard PJ. Microarray interrogation of human metanephric mesenchymal cells highlights potentially important molecules in vivo. *Physiol Genomics,* 23: 193-202, 2007.
3. Lye CM, Fasano L, Woolf AS. Ureter myogenesis: putting Teashirt into context. *J Am Soc Nephrol,* 21: 24-30, 2010.
4. Price KL, Woolf AS, Long DA. Unravelling the genetic landscape of urinary bladder development in mice. *J Urol,* 181: 2366-2374, 2009.
5. Adalat S, Bockenhauer D, Ledermann SE, Hennekam RC, Woolf AS. Renal malformations associated with mutations of developmental genes: messages from the clinic. *Pediatr Nephrol,* 11: 2242-2255, 2010.
6. Online Mendelian Inheritance in Man. John Hopkins University. http://www.ncbi.nlm.nih.gov/sites/entrez
7. Pitera JE, Scambler PJ, Woolf AS. Fras1, a basement membrane-associated protein mutated in Fraser syndrome, mediates both the initiation of the mammalian kidney and the integrity of renal glomeruli. *Hum Mol Genet,* 17: 3953-3964, 2008.
8. McGregor L, Makela V, Darling SM, Vrontou S, Chalepakis G, Roberts C, Smart N, Rutland P, Prescott N, Hopkins J, Bentley E, Shaw A, Roberts E, Mueller R, Jadeja S, Philip N, Nelson J, Francannet C, Perez-Aytes A, Megarbane A, Kerr B, Wainwright B, Woolf AS, Winter RM, Scambler PJ (2003) Fraser syndrome and mouse blebbed phenotype caused by mutations in FRAS1/Fras1 encoding a putative extracellular matrix protein. *Nat Genet,* 34: 203-208.
9. Ochoa B. Can a congenital dysfunctional bladder be diagnosed from a smile? The Ochoa syndrome updated. *Pediatr Nephrol,* 19: 6-12, 2004.
10. Daly SB, Urquhart JE, Hilton E,1 Edward A. McKenzie EA, Kammerer RA, Lewis M, Kerr B, Stuart H, Donnai D, Long DA, Burgu B, Aydogdu O, Derbent M, Garcia-Minaur S, Reardon W, Gener B, Shalev S, Smith R, Woolf AS, Black GA, Newman WG. Mutations in HPSE2 Cause Urofacial Syndrome. *Am J Hum Genet,* 86: 963-969, 2010.
11. Weber S, Thiele H, Mir S, Toliat MR, Sozeri B, Reutter H, et al. Draaken M, Ludwig M, Altmüller J, Frommolt P, Stuart HM, Ranjzad P, Hanley NA, Jennings R, Newman WG, Wilcox DT, Thiel U, Schlingmann K-P, Beetz R, Hoyer PF, Konrad M, Schaefer F, Nürnberg P, Woolf AS. Muscarinic acetylcholine receptor M3 mutation causes urinary bladder disease and a prune -belly-like syndrome. *Am J Hum Genet,* 89: 668-674, 2011.
12. Kerecuk L, Sajoo A, McGregor L, Berg J, Haq MR, Sebire NJ, Bingham C, Edghill EL, Ellard S, Taylor J, Rigden S, Flinter FA, Woolf AS. Autosomal dominant inheritance of non-syndromic renal hypoplasia and dysplasia: dramatic variation in clinical severity in a single kindred. *Nephrol Dial Transplant,* 22: 259-263, 2007.

13. Woolf AS. Environmental influences on renal tract development: a focus on maternal diet and the glucocorticoid hypothesis. *Klin Padiatr Supp,* l1: S10-S17, 2011.

14. Weber S, Moriniere V, Knuppel T, Charbit M, Dusek J, Ghiggeri GM, Jankauskiene A, Mir S, Montini G, Peco-Antic A, Wuhl E, Zurowska AM, Mehls O, Antignac C, Schaefer F, Salomon R Prevalence of mutations in renal developmental genes in children with renal hypodysplasia: results of the ESCAPE study. *J Am Soc Nephrol* ,17: 2864-2870, 2006.

15. Amiel J, Audollent S, Joly D, Dureau P, Salomon R, Tellier A.-L, Auge J, Bouissou F, Antignac C, Gubler M-C, Eccles MR, Munnich A, Vekemans M, Lyonnet S, Attie-Bitach T. PAX2 mutations in renal-coloboma syndrome: mutational hotspot and germline mosaicism. *Eur J Hum Genet* , 8: 820-826, 2000.

16. Adalat S, Woolf AS, Johnstone KA, Wirsing A, Harries LW, Long DA, Hennekam RC, Ledermann SE, Rees L, van't Hoff W, Marks SD, Trompeter RS, Tullus K, Winyard PJ, Cansick J, Mushtaq I, Dhillon HK, Bingham C, Edghill,EL, Shroff, R., Stanescu H, Ryffel GU, Ellard S, Bockenhauer D. HNF1B mutations associate with hypomagnesemia and renal magnesium wasting. *J Am Soc Nephrol* 20: 1123-1131, 2009.

17. Thomas R, Sanna-Cherchi S, Warady BA, Furth SL, Kaskel FJ, Gharavi AG. HNF1B and PAX2 mutations are a common cause of renal hypodysplasia in the CKiD cohort. *Pediatr Nephrol,* 26: 897-903, 2011.

18. Duke V, Quinton R, Gordon I, PMG Bouloux, Woolf AS. Proteinuria, hypertension and chronic renal failure in X-linked Kallmann's syndrome, a defined genetic cause of solitary functioning kidney. *Nephrol Dial Transplant,* 13: 1998-2003, 1998.

19. Duke V, Winyard PJD, Thorogood PV, Soothill P, Bouloux PMG, Woolf AS. KAL, a gene mutated in Kallmann's syndrome, is expressed in the first trimester of human development. *Mol Cell Endocrinol,* 110: 73-79, 1995.

20. Lazebnik N, Bellinger MF, Ferguson JE, Hogge JS, Hogge WA. Insights into the pathogenesis and natural history of fetuses with multicystic dysplastic kidney disease. *Prenat Diagn,* 19: 418-423, 1999.

21. Vissers LELM, van Ravenswaaij CM, Admiraal R, Hurst JA, de Vries BB, Janssen IM, van der Vliet WA, Huys EH, de Jong PJ, Hamel BC, Schoenmakers EF, Brunner HG, Veltman JA, van Kessel AG. Mutations in a new member of the chromodomain gene family cause CHARGE syndrome. *Nat Genet,* 36: 955-957, 2004.

22. Moreno-De-Luca D, SGENE Consortium, Mulle JG, Simons Simplex Collection Genetics Consortium, Kaminsky EB, Sanders SJ, GeneSTAR, Myers SM, Adam MP, Pakula AT, Eisenhauer NJ, Uhas K, Weik L, Guy L, Care ME, Morel CF, Boni C, Salbert BA, Chandrareddy A, Demmer LA, Chow EW, Surti U, Aradhya S, Pickering DL, Golden DM, Sanger WG, Aston E, Brothman AR, Gliem TJ, Thorland EC, Ackley T, Iyer R, Huang S, Barber JC, Crolla JA, Warren ST, Martin CL, Ledbetter DH. Deletion 17q12 is a recurrent copy number variant that confers high risk of autism and schizophrenia. *Am J Hum Gen,* 87: 618-630, 2010.

23. Draaken M, Reutter H, Schramm C, Bartels E, Boemers TM, Ebert AK, Rösch W, Schröder A, Stein R, Moebus S, Stienen D, Hoffmann P, Nöthen MM, Ludwig M. Microduplications at 22q11.21 are associated with non-syndromic classic bladder exstrophy. *Eur J Med Genet,* 53: 55-60, 2010.

24. Lambert HJ, Stewart A, Gullett AM, Cordell HJ, Malcolm S, Feather SA, Goodship JA, Goodship TH, Woolf AS; on behalf of the UK VUR Study Group. Primary, Nonsyndromic Vesicoureteric Reflux and Nephropathy in Sibling Pairs: A United Kingdom Cohort for a DNA Bank. *Clin J Am Soc Nephrol,* 6: 760-766, 2011.

25. van Eerde AM, Koeleman BPC, van de Kamp JM, de Jong TPVM, Wijmenga , Giltay CJC. Linkage study of 14 candidate genes and loci in four large Dutch families with vesico-ureteral reflux. *Pediatr Nephrol,* 22: 1129-1133, 2007.

26. Lu W, van Eerde AM, Fan X, Quintero-Rivera F, Kulkarni S, Ferguson H, Kim HG, Fan Y, Xi Q, Li QG, Sanlaville D, Andrews W, Sundaresan V, Bi W, Yan J, Giltay JC, Wijmenga C, de Jong TP, Feather SA, Woolf AS, Rao Y, Lupski JR, Eccles MR, Quade BJ, Gusella JF, Morton CC, Maas RL. Disruption of ROBO2 is associated with urinary tract anomalies and confers risk of vesicoureteral reflux. *Am J Hum Genet,* 80: 616-632, 2007.

27. Jenkins D, Bitner-Glindzicz M, Malcolm S, Allison J, Hu CC, Winyard PJ, Gullett AM, Thomas DF, Belk RA, Feather SA, Sun TT, Woolf AS. De novo Uroplakin IIIa mutations cause renal adysplasia leading to severe kidney failure. *J Am Soc Nephrol,* 16: 2141-2149, 2005.

28. Cordell HJ, Darlay R, Charoen P, Stewart A, Gullett AM, Lambert HJ, The UK VUR Study Group, Malcolm S, Feather SA, Goodship THJ, Woolf AS, Kenda RB, Goodship JA. Whole-genome linkage and association scan in primary, non-syndromic vesicoureteric reflux. *J Am Soc Nephrol,* 21: 113-123, 2010.

29. Köttgen A, Pattaro C, Böger CA, Fuchsberger C, Olden M, Glazer NL, Parsa A, Gao X, Yang Q, Smith AV, O'Connell JR, Li M, Schmidt H, Tanaka T, Isaacs A, Ketkar S, Hwang SJ, Johnson AD, Dehghan A, Teumer A, Paré G, Atkinson EJ, Zeller T, Lohman K, Cornelis MC, Probst-Hensch NM, Kronenberg F, Tönjes A, Hayward C, Aspelund T, Eiriksdottir G, Launer LJ, Harris TB, Rampersaud E, Mitchell BD, Arking DE, Boerwinkle E, Struchalin M, Cavalieri M, Singleton A, Giallauria F, Metter J, de Boer IH, Haritunians T, Lumley T, Siscovick D, Psaty BM, Zillikens MC, Oostra BA, Feitosa M, Province M, de Andrade M, Turner ST, Schillert A, Ziegler A, Wild PS, Schnabel RB, Wilde S, Munzel TF, Leak TS, Illig T, Klopp N, Meisinger C, Wichmann HE, Koenig W, Zgaga L, Zemunik T, Kolcic I, Minelli C, Hu FB, Johansson A, Igl W, Zaboli G, Wild SH, Wright AF, Campbell H, Ellinghaus D, Schreiber S, Aulchenko YS, Felix JF, Rivadeneira F, Uitterlinden AG, Hofman A, Imboden M, Nitsch D, Brandstätter A, Kollerits B, Kedenko L, Mägi R, Stumvoll M, Kovacs P, Boban M, Campbell S, Endlich K, Völzke H, Kroemer HK, Nauck M, Völker U, Polasek O, Vitart V, Badola S, Parker AN, Ridker PM, Kardia SL, Blankenberg S, Liu Y, Curhan GC, Franke A, Rochat T, Paulweber B, Prokopenko I, Wang W, Gudnason V, Shuldiner AR, Coresh J, Schmidt R, Ferrucci L, Shlipak MG, van Duijn CM, Borecki I, Krämer BK, Rudan I, Gyllensten U, Wilson JF, Witteman JC, Pramstaller PP, Rettig R, Hastie N, Chasman DI, Kao WH, Heid IM, Fox CS. New loci associated with kidney function and chronic kidney disease. *Nat Genet,* 42: 376-384, 2010.