AJKD

Case Report

Genomic and Epigenomic Analyses of Monozygotic Twins Discordant for Congenital Renal Agenesis

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Monozygotic twins have been widely studied to distinguish genetic and environmental factors in the pathogenesis of human diseases. For renal agenesis, the one-sided absence of renal tissue, the relative contributions of genetic and environmental factors to its pathogenesis are still unclear. In this study of a pair of monozygotic twins discordant for congenital renal agenesis, the genomic profile was analyzed from a set of blood samples using high-throughput exome-capture sequencing to detect single-nucleotide polymorphisms (SNPs), copy number variations (CNVs), and insertions and deletions (indels). Also, an epigenomic analysis used reduced-representation bisulfite sequencing to detect differentially methylated regions (DMRs). No discordant SNPs, CNVs, or indels were confirmed, but 514 DMRs were detected. KEGG analysis indicated the DMRs localized to 10 signaling pathways and 25 genes, including the mitogen-activated protein kinase pathway and 6 genes (*FGF18*, *FGF12*, *PDGFRA*, *MAPK11*, *AMH*, *CTBP1*) involved in organ development. Although methylation results from our adult patient and her sister may not represent the pattern that was present during kidney development, we could at least confirm a lack of obvious differences at the genome level, which suggests that nongenetic factors may be involved in the pathogenesis of renal agenesis.
Am J Kidney Dis. ■(■):■-■. © *2014 by the National Kidney Foundation, Inc.*

INDEX WORDS: Renal agenesis; monozygotic twins; genome; epigenome; congenital abnormalities of the kidney and urinary tract (CAKUT); methylation; nephrogenesis.

Congenital abnormalities of the kidney and urinary tract (CAKUT), which are defined as structural and functional malformations resulting in defective morphogenesis of the kidney and/or urinary tract,[1] occur in approximately 1 per 500 newborns and constitute approximately 20%-30% of anomalies found in neonates.[2,3] They are the most frequent cause of chronic kidney disease in children.[4,5] Renal agenesis is a common CAKUT[6,7] and is defined as the one-sided absence of renal tissue, which results from the failure of embryonic development of the metanephros or the absence of the nephrogenic cord.[8] Its cause is believed to be complex and may be affected by genetic and environment factors.[9]

In recent years, monozygotic twins have become an important experimental model to distinguish genetic and environmental factors in the pathogenesis of multifactorial diseases.[10,11] Monozygotic twins are presumed to be identical in genotype and different in phenotype due to environmental factors. Recent studies have found genetic and epigenetic differences between monozygotic twins.[12,13] Genetic differences may result from somatic mosaicism,[12] and environmental factors may contribute to the epigenetic changes, especially CpG methylation.[14]

In this study, single-nucleotide polymorphisms (SNPs), copy number variations (CNVs), insertions and deletions (indels), and differentially methylated regions (DMRs) were analyzed in a pair of monozygotic twins discordant for congenital renal agenesis. This analysis aimed to dissect the relative contributions of genetics and environment to the pathogenesis of renal agenesis.

CASE REPORT

A 30-year-old Chinese woman given a diagnosis of congenital renal agenesis of the left kidney at birth had a healthy monozygotic twin sister who did not have renal agenesis. The patient had no particular past or family history and no notable clinical symptoms. This study was conducted in accordance with the principles of the Declaration of Helsinki and has been reviewed and approved by the Ethics Committee of the Chinese PLA General Hospital (ethics approval number 2012-001). Written informed consent was obtained from both women.

To confirm that the twins were monozygotic, we analyzed short tandem repeat (STR) loci and amelogenin with a DNA sequencer (detailed methods for this and all other procedures described in this section are provided in Item S1, available as online

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Received October 21, 2013. Accepted in revised form January 6, 2014.

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0272-6386/$36.00

http://dx.doi.org/10.1053/j.ajkd.2014.01.423

supplementary material). As shown in Table 1, STR loci were consistent between the 2 samples, which suggests that this pair of twins was monozygotic.

Next we looked for SNPs that might differ between the patient and her twin. This analysis was done using the NimbleGen exome capture and sequencing system, followed by data analysis with GATK (Genome Analysis Toolkit[15]). As seen in Tables S1 and S2, we detected 97,039 SNPs in the patient and 97,814 SNPs in her twin; of these, 93,663 SNPs were identical between the 2 samples. First, as a check of the reliability of our SNP data, we compared results from our patient and her twin with 5 other twin pairs (Table S3). The genotype consistencies we observed in the twins in our study were within the range of other monozygotic twins and differed from data relating to a dizygotic twin pair. Thus, we concluded that the SNP results in this study are of acceptable accuracy. Next, we identified putative differential SNPs by determining the appropriate cutoff value denoting a substantial difference between the 2 samples in the fraction of reads supporting the variant allele (DiffSuppReads in Item S1). In all, we detected 30 putatively significant differential SNPs (Table S4); however, all were found to be false positives after manual inspection (Table S5).

We then analyzed the data using MuTect,[16] which usually is used to detect somatic point mutations in next-generation sequencing data of cancer genomes. These point mutations between paired samples are referred to as single-nucleotide variations (SNVs). In the patient and her sister, we detected 95 and 64 SNVs, respectively. However, only a small fraction (<5%) of the reads of the loci containing putative SNVs indicated a difference between the 2 samples (each locus is sequenced multiple times; only a low proportion of these reads gave a different sequence in one twin vs the other). This low mutation frequency means that there is a high possibility of the putative SNV being a false positive; these putative SNVs were discarded in subsequent data filtering. Thus, the MuTect analysis provided further evidence of a lack of single-nucleotide sequence differences between the 2 samples in this study.

Another way that genomes can differ is through CNVs. Using ExomeCNV,[17] we detected 3,535 focal CNVs; however, the CNVs did not significantly differ after CONTRA (copy number analysis for targeted resequencing) filtering[18] ($P = 0.3$ in the minimum target zone; Table S6), suggesting the absence of bona fide CNVs between the twins.

In addition, we used GATK to detect somatic indels. For this analysis, we defined the patient's sample as "normal" and her twin's as "tumor" (the process is intended for detecting indels that arise in tumor tissue). Sixty indels were detected by GATK, but all were deemed to be false positives after verification (Table S7).

To look for epigenomic differences between the patient and her sister, we focused on DMRs. We performed this analysis by reduced-representation bisulfite sequencing[19,20] (Fig 1; Tables S8 and S9). We then performed a KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis and found that 10 pathways and 25 genes were involved, as seen in Table S10. Among the 10 pathways, the mitogen-activated protein kinase (MAPK) pathway (including MAP kinase, JNK/p38 MAP kinase pathway, and ERK5 pathway) is well known to take part in the regulation of proliferation, differentiation, and apoptosis of cells. However, an association between the 9 other pathways and kidney development is not supported by existing evidence. Among the 25 genes, 6 (*FGF18*, *FGF12*, *PDGFRA*, *MAPK11*, *AMH*, and *CTBP1*) are involved in organ development, although there is no evidence that they are associated with kidney development.

Table 1. Short Tandem Repeat Typing Results for the Patient and Her Twin Sister

	Patient		Twin Sister	
Marker	Allele 1	Allele 2	Allele 1	Allele 2
AMEL	X		X	
D3S1358	15	15	15	15
D1S1656	16	16.3	16	16.3
D6S1043	12	15	12	15
D13S317	10	11	10	11
Penta E	12	20	12	20
D16S539	10	11	10	11
D18S51	12	14	12	14
D2S1338	18	24	18	24
CSF1P0	11	12	11	12
Penta D	9	11	9	11
THO1	7	9	7	9
vWA	14	17	14	17
D21S11	29	32.2	29	32.2
D7S820	9	10	9	10
D5S818	11	13	11	13
TPOX	8	11	8	11
D8S1179	13	15	13	15
D12S391	18	21	18	21
D19S433	13	13	13	13
FGA	23	26	23	26

Note: Analysis performed using a human identification kit that amplifies 20 short tandem repeat loci and the amelogenin locus (for sex typing). Values shown are the number of short tandem repeats detected at each allele of each locus.

DISCUSSION

This study had 2 innovative aspects. First, cases of monozygotic twins discordant for congenital renal agenesis are very rare. To our knowledge, this study constitutes the first analysis of SNPs, CNVs, indels, and DMRs to directly observe the differences resulting from genetic and environmental factors in monozygotic twins discordant for congenital renal agenesis. Second, this study provides evidence suggesting a lack of differences at the sequence level (SNPs, CNVs, and indels) and the presence of epigenetic differences (at least in terms of differential methylation) in a pair of monozygotic twins who were discordant for congenital renal agenesis. Thus, we suggest that environmental factors, not genetic factors, may lead to the pathogenesis of renal agenesis by epigenetic changes.

In week 5 of gestation, the ureteric bud, originating from the Wolffian duct, penetrates the metanephric blastema. After 2 further weeks of gestation, the ureteric bud induces nephrogenesis. By week 20, the ureteric bud has branched and created the collecting duct system in its entirety. However, at that stage, nephrogenesis is just 30% complete, and there is further maturation until week 36.[21] Because methylation changes over time, the DMRs detected in this study of 30-year-old twins may not represent the