Mutations in the RARE and MARE regulatory sequences of HNF1β are not a frequent cause of kidney/urinary tract malformation

Sir,

Mutations in several genes have been identified in congenital abnormalities of kidneys and urinary tract (CAKUT): heterozygous mutations in HNF1β account for 8%, while other genes (PAX2, EYA1, SALL1, SIX1) have been identified as causing isolated renal hypo/dysplasia to a lesser extent in humans [1].

Despite extensive HNF1β molecular analysis, including direct sequencing of the minimal promoter and of the nine exons associated with screening for exonic rearrangement, HNF1β genetic testing failed to identify mutations in a majority of patients with CAKUT, even in individuals with very evocative renal phenotype [2,3]. Recently, specifically conserved cis-acting RARE (retinoic acid responsive element) and T-MARE (MafB responsive element) regulatory sequences have been identified [4]. RA signalling cascade and MafB bind RARE and T-MARE regulatory DNA sequences, respectively, and promote the expression of Hnf1β in both the caudal hindbrain (r4/r5 boundary) and pancreas. These sequences are both located in intron 4 of HNF1β and are not actually studied for the purpose of diagnosis. Finally, the roles of RA and MAFB in kidney development suggest that the RA-MAFB-HNF1β pathway may also be functional in human kidney.

In this study, we hypothesized that mutations in the highly conserved cis-acting RARE and T-MARE regulatory sequences could disturb the early embryologic process of intermediate mesoderm, from which kidneys and urinary tract both derived, and consequently lead to renal phenotype mimicking HNF1β-related nephropathy.

Among the 63 individuals we tested for HNF1β, a mutation was identified in 12 (19%). We therefore performed molecular analysis of these regulatory sequences in the 51 remaining patients with isolated or syndromic CAKUT of unknown origin for whom previous molecular analysis of HNF1β (QMPSF and direct sequencing) had failed to identify any causative mutation. All patients gave informed consent according to French law.

Renal and extra-renal features of the 51 tested patients [31 males, 20 females, median age 9 years (0–53)] are summarized in Tables 1 and 2, respectively. RARE and T-MARE regulatory sequences were amplified by PCR using a set of primers deduced from the genomic sequence (forward: TC CCCAGAACCCTTCCTCTCCA, reverse: TGGTCAAAGCC CCAAATGTAAATGGT), corresponding to amplification of a 344 bp fragment (G1266–17138 to G1266–16794). Among the 51 tested patients, no sequence variation of the RARE sequences (boxes). Dashes indicate conserved residues.

Table 1. Renal characteristics of 51 patients with CAKUT tested for RARE and T-MARE regulatory sequences of HNF1β

| Renal features                  | N   |
|--------------------------------|-----|
| Hyperechogenicity               | 16  |
| Renal cysts                     | 33  |
| Kidney size                     |     |
| Normal                          | 27  |
| Enlarged                        | 4   |
| Unilateral hypoplasia           | 8   |
| Bilateral hypoplasia            | 12  |
| Urinary tract malformation      |     |
| Bilateral VUR                   | 6   |
| Solitary kidney                 | 5   |
| Ectopic kidney                  | 3   |
| UPJO                            | 1   |
| Oligomeganephronia              | 1   |
| Chronic renal failure           | 17  |

Table 2. Extra-renal characteristics of 51 patients with CAKUT tested for RARE and T-MARE regulatory sequences of HNF1β

| Extra-renal features            | N   |
|---------------------------------|-----|
| Genital tract                   |     |
| Cryptorchidism                  | 2   |
| Bicornual uterus                | 1   |
| MRKH syndrome                   | 1   |
| Vas deferens absence            | 2   |
| Testis + semen vesicle hypoplasia| 1   |
| Liver                           |     |
| Abnormal liver tests            | 1   |
| Choledochal cyst and DM         | 1   |
| Pancreas                        |     |
| Chronic calcified pancreatitis and DM | 2 |
| Mental retardation              | 1   |
| Retinal oedema                  | 1   |

CAKUT: congenital abnormalities of kidneys and urinary tract; VUR: vesico-ureteric reflux; UPJO: uretero-pyelic junction obstruction.

MRKH: Mayer-Rokitansky-Küster-Hauser; DM: diabetes mellitus.
and T-MARE regulatory sequences of HNF1β was evidenced.

In conclusion, although mutations in RARE and T-MARE regulatory sequences of HNF1β may nevertheless be implicated in some renal congenital disorders, we show here that mutations in these sequences are not a frequent cause of CAKUT. More experiments are required to assess the role of the RA-MAFB-HNF1β pathway in kidney development. Moreover, the molecular basis of these renal malformations is still poorly understood, and further works remain to be done to identify new CAKUT genes. Delination of transcriptional networks involved in early human metanephros development may be a way to identify these new genes.

Conflict of interest statement. None declared.

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PC = [Cd_{out} - (1-D/Qd_{\text{in}}) \times Cd_{\text{in}}]/(D/Qd_{\text{in}}),

where Qd_{\text{in}} and Qd_{\text{out}} are dialysate flow at, respectively, inlet and outlet, Cd_{\text{in}} and Cd_{\text{out}} are dialysate conductivity at, respectively, inlet and outlet and D is ionic dialysance, and

\text{IMB} = (Qd_{\text{in}} \times Cd_{\text{in}} - Qd_{\text{out}} \times Cd_{\text{out}}) \times 10 \times \text{time(min)}.

A positive IMB means sodium removal from the patient. A negative IMB means sodium transport to the patient.

In patients with zero inter-diaytic weight gain, IMB was measured in 200 isovolaemic haemodialysis sessions by Diascan®, 137 sessions were performed with four to eight conductivity pulses. A total of 63 sessions was performed with only one conductivity pulse. The results showed a highly significant correlation between pre-dialytic plasma conductivity and IMB (Spearman rank $r_s = 0.902$, $P < 0.005$), in agreement with previous studies [6]. There was no correlation between IMB and the number of conductivity pulses (Spearman rank $r_s = 0.02$, $P = 0.328$) (Figure 1).

Fig. 1. Correlation between IMB during isovolaemic haemodialysis and number of conductivity pulses.