A MEN2A family with two asymptomatic carriers affected by unilateral renal agenesis

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Abstract. Accumulating evidences suggest RET gene’s involvement in development of the kidney in mice and humans. Although it is well known that RET mutation causes multiple endocrine neoplasia type 2A (MEN2A), thus far only 3 individuals have been reported to have MEN2A and renal agenesis/dysgenesis. We report a MEN2A family with RET mutation in which two asymptomatic carriers presented with unilateral renal agenesis. A 48-year-old woman underwent total thyroidectomy with regional lymph node dissection in our department for medullary thyroid carcinoma. She had earlier surgical treatment for a left adrenal pheochromocytoma at the age of 45. In the screening for MEN type 2 for her three sons, a CT scan for adrenal pheochromocytoma incidentally found unilateral renal agenesis in two of the sons, one of whom had suffered from Hirschsprung’s disease (HSCR). They had contralateral kidneys exhibiting compensatory hypertrophy and normal renal function. Genetic analysis detected C618R RET mutation in the proband and her 3 sons, and no other mutations were found in RET as well as glial cell line-derived neurotrophic factor (GDNF). Our data lend support to the hypothesis that constitutive active RET mutation in MEN type 2 might partially impair RET function and thereby cause loss of function phenotype such as renal agenesis or HSCR.

Key words: RET mutation, Renal agenesis, MEN2, Hirschsprung’s disease

THE RET GENE encodes a member of the receptor tyrosine-kinase family that has been implicated in the normal development and neoplastic growth of neural crest lineages [1-3]. It is well-known that germline defects of this proto-oncogene develop multiple endocrine neoplasia type 2A (MEN2A) and familial medullary thyroid carcinoma (FMTC) [4-7]. Likewise, the germline mutations are also responsible for familial cases of Hirschsprung’s disease (HSCR), which is a congenital abnormality pathologically characterized by an absence of intrinsic ganglion cells of the distal gastrointestinal tract, resulting in functional intestinal obstruction in neonates and children [8-10]. In a small fraction of kindreds, MEN2/FMTC cosegregates with HSCR [11-15], although the developmental mechanisms of MEN2/ FMTC and HSCR are quite different, because MEN2/ FMTC and HSCR are caused by a gain-of-function and a loss-of-function mutations, respectively [16, 17].

Apart from HSCR, kidney dysplasia is also an anomalous development reported to be involved in the loss of function of RET mutation. An experimental study indicated Ret function as an essential component of enteric neurogenesis and renal organogenesis, by its exhibition loss of enteric neurons in addition to renal agenesis or severe dysgenesis in Ret knockout mice [18]. The observation of a high level of RET expression in urine bud of fetal was reported, indicating that RET expression may be involved in the course of human kidney development [19]. Indeed, mutations in the RET gene have been reported in fetuses with renal agenesis [20, 21]. Interestingly, renal agenesis/dysgenesis in the MEN2A family associated with RET mutation has been reported in only three living individuals of two kindreds [22, 23]. We present herein a kindred affected by RET mutation in which two members manifested unilateral renal agenesis, one of whom
also presented HSCR. The mutation of RET, and glial cell line-derived neurotrophic factor (GDNF), which is a ligand of RET, were also studied in this family.

**Case Report**

A 47-year-old female presented at our hospital with neck tumor. Diagnostic images showed multiple nodules in her thyroid gland and fine needle biopsy for these tumors indicated medullary thyroid cancer. Her medical history included a left adrenalectomy at the age of 44, which histopathological examination revealed to be pheochromocytoma. She underwent total thyroidectomy and pathological examination confirmed medullary thyroid cancer. Coexistence of pheochromocytoma and medullary thyroid carcinoma suggested that this patient had MEN type 2. Therefore, genetic analysis was performed and detected a C618R mutation in RET gene. Thus, she was diagnosed as proband of MEN2A. The pedigree of her family is shown in Fig. 1. She has three sons. Member III-3 had a history of HSCR and underwent surgical treatment 3 months after birth. We performed a screening for MEN2A in her 3 sons, but neither laboratory data nor image diagnoses (neck US and abdominal CT scan) suggested that they presented MTC, pheochromocytoma or primary hyperparathyroidism (Table 1). However, the CT scan showed unilateral agenesis and contralateral hypertrophy of the kidneys of two of the boys (Figs. 2, 3). The laboratory data on renal function exhibited normal (Table1).

Her three sons were subjected to pre-symptomatic genetic testing after appropriate informed consent was obtained and all of them were found to carry the same C618R mutation. To search for the variant associated with the renal agenesis, the entire coding region of the RET gene of the proband, her husband and sons was surveyed by Sanger sequencing (approved by the Ethical Review Board for Human Genome Studies at Fujita Health University). We found two common synonymous variations, c.135A>G (A45A) (rs1800858) and c.1296A>G (A432A) (rs1800860), linking to the C618R mutation, but no possible loss-of-function mutation was identified. In addition, we genotyped a potential modifier variant within the enhancer-like element located in the intron 1 (rs2435357) [24]. The C618R mutation was found to link to T allele (Fig. 1), which reportedly reduced the enhancer activity. Further, we also surveyed the entire coding region of the GDNF gene, but no mutation was found.

**Table 1** Laboratory data related to MEN2A and renal function

<table>
<thead>
<tr>
<th>Member</th>
<th>III-1</th>
<th>III-2</th>
<th>III-3 (normal range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcitonin (pg/mL)</td>
<td>39</td>
<td>38</td>
<td>23 (15-86)</td>
</tr>
<tr>
<td>CEA (ng/mL)</td>
<td>2.3</td>
<td>1.5</td>
<td>1.2 (0-5.0)</td>
</tr>
<tr>
<td>intact PTH (pg/mL)</td>
<td>38.1</td>
<td>53.7</td>
<td>46.4 (15.0-68.3)</td>
</tr>
<tr>
<td>Ca (mg/dL)</td>
<td>9.8</td>
<td>9.2</td>
<td>9.6 (8.7-10.3)</td>
</tr>
<tr>
<td>Total metanephrine (mg/day)</td>
<td>0.54</td>
<td>0.31</td>
<td>0.75 (0.13-0.52)</td>
</tr>
<tr>
<td>Cr (mg/dL)</td>
<td>0.74</td>
<td>0.70</td>
<td>0.77 (0.6-1.1)</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73m²)</td>
<td>101.6</td>
<td>110.0</td>
<td>101.4 (90-)</td>
</tr>
</tbody>
</table>

**Discussion**

In the development of kidney, the Wolffian duct forms from the intermediate mesoderm and grows caudally, first inducing tubules of the pronephric and mesonephric kidneys, and later giving rise at its caudal end to the ureteric bud, a major component of the metanephric or permanent kidney [25]. In an earlier
Fig. 2 CT scanning showed right renal agenesis and compensatory hypertrophy of contralateral kidney (arrow) in member III-1.

Fig. 3 CT scanning showed left renal agenesis and compensatory hypertrophy of contralateral kidney (arrow) in member III-3.
study, Schuchardt et al. demonstrated that Ret gene is expressed in the Wolffian duct and ureteric bud epithelium of mice, and that Ret knockout mice exhibit renal agenesis/dysgenesis, indicating the participation of the gene in kidney development [19]. Afterward, many reports described the crucial need for RET/GDNF signaling in renal branching morphogenesis.

In a recent study of 33 stillborns with renal agenesis/dysgenesis, Skinner et al. reported a high incidence of RET mutation in 7 of 19 fetuses, presenting with bilateral renal agenesis (37%), and 2 of 10 fetuses had unilateral agenesis (20%) [20]. However, in another study of 105 stillborn fetuses with bilateral renal agenesis/dysgenesis, Jeanpierre et al. identified a low frequency (< 7%) of potential RET mutations [21]. Thus, although the low prevalence of renal agenesis/dysgenesis caused by RET mutation remains unclear, it would be extremely rare in living individuals. To date, there have been only 2 reports describing renal agenesis/dysgenesis associated with RET mutation [22, 23]. First, Loré et al. reported 2 members (a 32-year-old woman and her son) with unilateral renal agenesis in a kindred affected by RET mutation. The woman manifested FMTC and left renal agenesis, and the son presented HSCR and left renal agenesis. C620S RET mutation was detected in both of them [22]. Second, McIntyre et al. reported a 29-year-old man with the full spectrum of MEN2A (medullary thyroid carcinoma, pheochromocytoma and primary hyperparathyroidism), in addition to right hydronephrosis requiring right pyeloplasty with reimplantation of the ureter in the bladder apex, and severe left renal dysgenesis, carrying C634A RET mutation [23]. Thus, a total of 3 cases with RET mutation have been presented, suggesting that a penetration of renal agenesis/dysgenesis is extremely rare compared with the number of cases with MEN2 or FMTC. Indeed, Lambardo et al. reported a review of 232 patients belonging to 72 families with inherited medullary thyroid carcinoma who carried a RET germline mutation, but found no cases with renal agenesis/dysgenesis among them [26].

As mentioned above, renal agenesis/dysgenesis is extremely rare in cases with RET mutation. Therefore we analyzed an abnormality of the GDNF gene, which is one of the ligands of RET receptors, or other mutations in RET gene. Although Moore et al. reported that Gdnf knockout mice lack the development of normal kidneys as in Ret knockout mice [27], and furthermore, that consequential experimental studies demonstrated the involvement of Gdnf gene in normal kidney development, the gene mutation was not detected in our cases.

One member in the kindred presented not only unilateral renal agenesis, but HSCR. Approximately 20% of the cases with HSCR have been familial, and the abnormality of 10 genes, including RET gene and five loci, have currently been described as being involved in HSCR development [28]. Schuchardt et al. demonstrated that Ret knockout mice manifested a lack of enteric neurons throughout the digestive tract, aside from renal agenesis/dysgenesis [18]. Indeed, RET gene mutation was found in HSCR patients [8, 9]. Furthermore, in a small fraction of kindreds, cosegregation of MEN2/FMTC and HSCR were described [11-15]. Only one individual so far was reported to have a co-occurrence of unilateral renal agenesis and HSCR with RET gene mutation [22]. Member III-3 is the second case who additionally supported that the co-occurrence would not result from a coincidence, but from an involvement in RET gene mutation.

Recently, C>T variation within the enhancer-like element located in the intron 1 (rs2435357) was reported to affect the contribution of the RET mutation to the onset of HSCR [24]. T-allele increases the disease penetrance in HSCR patients with RET coding mutation [29]. In our cases, C618R mutation was found to link to the T-allele. This suggests that the lower enhancer activity of the T-allele instigated the loss-of-function nature of the C618R mutation leading to the onset of not only HSCR in III-3 but also renal agenesis in III-1 and III-3. Further, since the transmission frequency of this allele was found to be smaller to affected daughters than to affected sons, this modifier effect appears stronger in a case of transmission to male than to female [24]. The fact that both of the presented cases are male may be supported by this observation.

In conclusion, although clinical renal agenesis/dysgenesis in association with MEN type 2/FMTC was extremely rare, as illustrated in the present cases, a 618 RET mutation can cause not only HSCR but renal developmental abnormality by the loss-of-function nature of RET mutation.

Conflict of Interest

All authors have no conflicts of interest.
References


