RESEARCH NOTE

Collectrin gene screening in Turner syndrome patients with kidney malformation

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Introduction

Turner syndrome (TS) affects one in 2500–3000 live-born girls, and is the most prevalent female sex chromosomal disorder in humans, resulting from the loss of all or part of one of the two X chromosomes (Sybert and McCauley 2004). About 50% of TS patients carry a 45, X monosomy, the rest being mosaics or structural chromosome abnormalities. TS patients' phenotype is variable, and the wide spectrum of clinical features includes: short stature, ovarian dysgenesis, lymphedema, cardiovascular defects and renal malformation. Diabetes mellitus is also present at a rate two to four times higher than in the general population (Elsheikh *et al*. 2002), consistent with the finding of a relative insulin deficiency in TS on a non-immune basis, suggesting that haploinsufficiency for X-chromosome gene(s) impairs beta-cell function predisposing to diabetes mellitus in TS (Bakalov *et al*. 2004). The loss of the short arm of chromosome X (Xp), common to all TS, generally results in the full syndrome phenotype (Elsheikh *et al*. 2002).

So far, a correlation between karyotype and phenotype in TS patients has not been clearly defined. Haploinsufficiency of several genes mapped to the short arm of X chromosome has been related to the clinical signs observed in TS patients; for example, the *SHOX* (short stature homeobox-containing gene) responsible for the short stature characteristic of the patients.

Association of renal abnormalities and TS varies from 33% to 70% (Matthies *et al*. 1971). These include horseshoe kidney, duplication of collecting system, ectopic kidney, ureteropelvic stenosis, renal agenesis and renal cysts. Two forms of cystic kidney disease have been described in TS: multicystic dysplastic kidney (MCDK) and simple renal cysts (Fanos *et al*. 2000).

In order to identify a genotype–phenotype correlation in TS, focussing on kidney abnormalities and diabetes, we previously sequenced the hepatocyte nuclear factor 1beta (*HNF1B*) gene in a cohort of TS patients carrying the renal phenotype. No mutation on the coding sequence of the gene was identified (D'Amato *et al*. 2007). The role of *HNF1B* in kidney development and function has been previously reported (Igarashi *et al*. 2005). Moreover *HNF1B* heterozygous mutation results in the maturity-onset diabetes of the young type 5 (MODY 5) disease with a phenotype that associates diabetes mellitus and renal abnormalities (such as cystic dysplasia and abnormal nephron development). MODY 5 may also affect females with internal genital abnormalities.

TMEM27 gene, a novel target of HNF1complex (HNF1β-HNF1α heterodimer or HNF1α, HNF1β homodimer), was recently identified and studied in animal models (Akpinar *et al*. 2005; Fukui *et al*. 2005; Danilczyk *et al*. 2006). The gene, located on the short arm of X chromosome, encodes a tissue specific protein termed collectrin (Zhang *et al*. 2001), now known to be expressed predominantly and almost exclusively in pancreatic beta cells and tubular ducts of the kidneys. *TMEM27* is a non-pseudoautosomal gene and, thus, does not have a Y-chromosome homologue; in TS, as in male subjects, only one copy of the gene is present.

In the present study, we attempt to test whether alteration in *TMEM27* gene is related to kidney malformations observed in TS subjects. We, therefore, recruited 18 TS patients definitely missing one copy of the short arm of X chromosome (Xp), with and without kidney malformations. *TMEM27* coding sequence, minimal promoter and 3′UTR regions were sequenced in these patients. This is the first human study of the gene *TMEM27* and an attempt to shine a light on genotype–phenotype correlation in TS patients.

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Materials and methods

Subjects

Fourteen TS patients carrying kidney malformations, previously described by our group (D'Amato *et al*. 2007) were re-evaluated. All 14 patients (cases) were recruited from Department of Paediatrics, Endocrinological Section, Policlinico San Matteo, Pavia, Italy. In addition four patients recruited from the Department of Paediatrics, Endocrinological Section, Institute G. Gaslini, Genoa, Italy, noncarriers of kidney malformations, were used as control cases (table 1). TS was diagnosed by karyotyping. Mean age of cases at evaluation was 18 yr and 5 months (range 8/5–34/11 years/months) and controls mean age 18 yr (range 4/1–30/3 years/months). Kidney morphological evaluation, detected by ultrasound, revealed horseshoe kidney in 11 patients, renal agenesis in two and renal cysts in one, while no kidney morphological alteration was observed in all four control cases (table 1). Family history for kidney abnormalities was negative for all but 1 case, showing a renal agenesis in the patient and in the paternal grandfather. Type 2 diabetes family histories were positive for four cases, while type 1 diabetes was absent in all. Patients underwent oral glucose tolerance test in order to assess glucose metabolism. Normal fasting blood glucose was observed in all cases, while impaired glucose tolerance was found in three cases. Immunological markers of type 1 diabetes (GADA IA-2A and IAA) were absent in all cases. Control case patients did not have glucose metabolism alterations.

Methods

Genomic DNA was obtained from peripheral blood samples. The six exons composing *TMEM27* gene, including the intron–exon boundary regions were amplified by PCR. Additionally a minimal promoter region of 260 bp upstream the coding sequence, where binding sites for HNF1 complex were previously identified (Akpinar *et al*. 2005; Fukui *et al*. 2005), and the 3′UTR region, were also amplified. PCRs were performed in Perkin Elmer GeneAmp PCR System 9700 and the cycling conditions are as follows: initial denaturation at 94°C for 5 min, 30 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 7 min. Primers sequences used are as follows: exon 1 forward: 5′-TGGTGGCTCGCTCGTTTC-3′, reverse: 5′-ACTGCCCCAACTTTCAAGC-3′; exon 2, forward: 5′-CAGGAGTATTTGGGGCTGTT-3′, reverse: 5′-TGCAGACAGGCCCATTATTAG-3′; exon 3 forward: 5′-GGAGAGCCACTGTGGGTACA-3′, reverse: 5′-AAAGTGTGAATCCCTTTGAAAAA-3′; exon 4 forward: 5′-TGATTTTGAAACAATGGGAATTT-3′, reverse: 5′-TGCCCCTGGATGAGAACTAC-3′; exon 5 forward: 5′-GCCAGGAGGATGCTTTGTT-3′, reverse: 5′-GGAAAATCCTCTCCTGATTTG-3′; exon 6 forward: 5′-TGGTCTTTGAAATTCGTTTGA-3′, reverse: 5′-TTCAGTGGTGTTGGTGGGTA-3′; for the promoter region: forward 5′-CCAGGTATCTCAGCCTCGAA-3′; reverse 5′-GGTGGAAAACACAAGGCAAA-3′ and for the 3′UTR region: forward 5′-

Table 1. Clinical and genetic characteristics of the enrolled patients.

Case number	Age (years/months)	Karyotype	Kidney malformation	Family history for renal pathology
1	15/6	45,X/46,X,i(Xq)	1	Neg
2	15/7	45,X	1	Neg
3	15/10	45,X	1	Neg
4	15/11	45,X	1	Neg
5	16/1	45,X	1	Neg
6	17/2	45,X	2	Pos
7	17/6	45,X/46,X,del(Xq)	1	Neg
8	17/9	45,X	1	Neg
9	18	45,X/46,XX	1	Neg
10[a]	18/7	45,X/47,X,i(Xq),i(Xq)	1	Neg
11[a]	22	45,X/46,XX	3	Neg
12[a]	26/1	45,X	1	Neg
13	34/11	45,X	2	Neg
14	8/5	45,X	1	Neg
15	15/6	45,X/46,XX	Neg	Neg
16	4/1	45,X	Neg	Neg
17	30/3	45,X/46,XX	Neg	Neg
18	22/3	45,X	Neg	Neg

Kidney malformation included horseshoe kidney (1), kidney agenesis (2), and polycystic kidney (3); Neg, negative, Pos, positive; [a]impaired glucose tolerance.

TGCTTGAAAGTGAAAAGCAATC-3′; reverse 5′-TCCAGACCACAATCAGTCACA-3′. PCR products were purified using ExoSoap enzyme and both strands were sequenced using a BigDye Terminator Cycle Sequencing Kit (PE Biosystems) according to the manufacturer's recommendations. Reactions were analysed on an ABI PRISM 310 DNA Sequencer (PE Biosystems).

Results and discussion

Direct sequencing of the six exons, including intron–exon boundary regions, minimal promoter region containing the binding sites for HNF1 complex, and 3′UTR region of the *TMEM27* gene, did not show any nucleotide changes leading to gene mutation. Of the total 18 TS subjects screened (14 probands and four controls), only one polymorphic nucleotide change, corresponding to SNP rs5936000, was found in three probands: 2, 6 and 13 who carried the G instead of the A allele in hemizygous state. *HNF1B* gene was also previously sequenced on the same cohort of patients (D'Amato *et al*. 2007), but no relevant nucleotide change was evidenced.

Phenotype–genotype correlation is hard to investigate in TS, and many etiopathogenetic factors may play a role in such a complex and heterogeneous phenotype. Renal abnormalities in TS include a large spectrum of malformation; while positional abnormalities observed may reflect varying degrees of failure of the migratory process observed during the embryonic development (Matthies *et al*. 1971). Others, such as renal aplasia may suggest an underlined embryologic malformation in budding of the metanephros. A widely diffuse hypothesis proposed by Ogata and Matsuo (1995) involves lymphatic stasis secondary to lymphatic hypoplasia resulting in enlarged lymphatic vessels and a consequent compression of organ systems. According to this hypothesis, the developmental renal malformation is the result of distended retroabdominal and iliac lymphatic vessels inhibiting rotation and migration of the kidneys (Ogata and Matsuo 1995). This elegant hypothesis could explain positional and some structural renal anomalies occurring in TS patients exhibiting lymphedema, but remains difficult to prove and so far identification of a putative lymphogenic gene is still obscure. In particular, renal cystic disease in TS pathogenesis is not yet clarified. While an association of cardiovascular defects in TS with foetal lymphedema has been demonstrated (Loscalzo *et al*. 2005), the pathogenesis of kidney abnormalities in TS has not yet been clarified.

In non-TS population, horseshoe kidney occurs in approximately one in 400 people and it appears twice as common in men as in women. (O'Brien *et al*. 2008). No causative genetic determinant is known, although the malformation is reported in identical twins and in siblings within the same family. MCDK is also found with a higher prevalence in males (Merrot *et al*. 2006). The incidence of kidney malformation in TS is thereby closer to that found in the male general population than in females. We speculate that mutations affecting a nonpseudoautosomal gene on the X chromosome may be responsible for the renal phenotype in the female TS patients; the same gene would in fact be expected to cause a similar phenotype with a similar incidence in males who are naturally hemizygous for non-pseudoautosomal X chromosome genes.

HNF1B mutations result in MODY-5, a disease characterized by the association of alteration of the glucidic metabolism and renal abnormalities (Bellanne-Chantelot *et al*. 2004). In a previous work we directly sequenced the same cohort of patients analysed in this work for *HNF1B* gene (D'Amato *et al*. 2007).

Herein, we analyse a recently identified novel target of HNF1B, a non-pseudoautosomal gene on the short arm of X chromosome. Fukui *et al*. (2005) described the implication of this molecule (collectrin) in the exocytosis of insulin granules in beta-cells by binding other proteins and taking part in the constitution of the SNARE complex (Fukui *et al*. 2005). Other authors showed that inhibition of collectrin results in renal phenotype with lack of cilium and cystic formation, using siRNA experiments (Zhang *et al*. 2007). So far, to our knowledge, the gene has not yet been studied in primates and no data is available on humans.

In the present study, the absence of mutations in the whole translated sequence, in the minimal promoter region, inclusive of the HNF1 complex binding sites, and in the 3′UTR region, of the *TMEM27* gene, allow us to exclude any direct mutation of the gene to be causative of the phenotype. Although we sequenced the exon–intron boundary regions, where a significant number of SNPs are present (rs5936000, rs10613600, rs12833789, rs1055316 and rs6653979), we only found an hemizygous polymorphic change in intron 4: SNP rs5936000 (G to A) in patients 2, 6 and 13. We could not, however, correlate this polymorphic change with any phenotypic features because of the small number of patients analysed, and because of the different phenotypes showed by the subjects carrying the polymorphic change. In fact, patient 2 is affected by horshoe kidney, whereas patients 6 and 13 are affected by kidney agenesis. We cannot, however, exclude gene expression alteration due to possible further polymorphic variations present in the large introns of the gene. Moreover, epigenetic events could also have influenced the TS patient phenotypes; for example CpG island methylation may control *TMEM27* expression during the kidney developmental timeline. Thus, *TMEM27* gene expression may still be worth evaluating in TS patients carrying a kidney phenotype.

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