Expression analysis of RBMY1, CDY1, and VCY2 genes in Korean male infertility

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Azoospermia factor (AZFa, b, and c) regions have been focused on their involvement in the spermatogenic process by frequent observation of microdeletion in male infertility. Among the azoospermia factors, RBMY1, CDY1, and VCY2 genes are strongly associated with the male germinal cell differentiation and development in testis. Using RT-PCR approach, expression patterns of RBMY1, CDY1, and VCY2 genes are examined in testicular biopsy specimens from 42 Korean azoospermic patients. No expression of RBMY1, CDY1, and VCY2 genes appeared as 34%, 66%, and 27% of the male infertility, respectively. Patients who had no expression of RBMY1 and VCY2 genes also showed negative expression of the CDY1 gene in their testis tissues. All Sertoli cell-only syndrome patients showed no expression of the CDY1 gene. Taken together, the CDY1 gene expression seems to be necessary factor to complete spermatogenesis in Korean population.

Key words – CDY1, RBMY1, VCY2, Korean population, male infertility

Introduction

Infertility is a big problem in seeking their children after marriage, and 10-15% of couples suffered from that situation. In half of the infertility couples, main causes of infertility are in man. Compared to female infertility, male infertility is very complex disease related to the malnutrition, endocrinological disorders, genetic factors, and environmental hazards [11]. The genetic factors such as microdeletion and chromosomal abnormalities have been estimated to account for at least 30% of male infertility [1].

Tiepolo and Zuffardi (1976) demonstrated that Yq11 region defined as "azoospermia factor (AZF)" is very important to complete the spermatogenesis by the investigation of microscopically detectable deletions in azoospermic patients [22]. This region had been further divided into three non-overlapping regions of AZFa, AZFb and AZFc [9,25]. Among these regions, the AZFc region was highlighted by the frequent microdeletions, comprising about 80% of all detected microdeletions [14,27]. Recently, some of genes on AZF regions were reported to be candidate genes which could affect the male germ cell differentiation, development, and specifically male infertility [17]. RBMY1, CDY1 and VCY2 (BPY2) genes in AZFc and AZFb have been examined by the microdeletion analysis in DNA and protein levels in western population [2,3,8,11,15,26].

RBMY (RNA Binging Motif Y chromosome) was identified as a major candidate gene related to spermatogenesis [16,24]. Although six RBMY subfamilies are divided into the current Y chromosome (RBMY1 to RBMY6) by the unknown process, RBMY1 is only thought to be the actively transcribed genes in human testicular tissues. The RBMY1 gene is expressed specifically in spermatogonia and early primary spermatocyte stages during the spermatogenesis [4,8,19]. RBM gene families were known to regulate RNA processing and provide germ cell-specifc components with hnRNPGT and T-STAT proteins [8].

CDY (Chromodomain on Y chromosome) gene was noticed by the association of Y chromosomal microdeletions detected in infertile men. The CDY gene has been evolved from the Y chromosomal retrotransposition of a transcript generated from the autosomal single copy CDYL (CDY-like) gene located on chromosome 6, and acquired their specific function in male testis by the functional diversification [12,19]. Thereafter, CDY underwent gene duplication on the Y chromosome (CDY1 and CDY2). The CDY1 tran-

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scripts play an important role to complete spermatogenesis and expressed elongated spermatids in late stages of spermatogenesis after meiosis [13].

VCY2 (Variable charge Y chromosome 2; alias BPY2) gene is located in the AZFc region where frequently deleted in azospermic patients. During the spermatogenesis, VCY2 gene expression was detected at the spermatogonia, spermatocytes, round spermatids, and ejaculated spermatozoa stages in the testicular biopsy tissues by the immunobiochemical analysis [23]. The VCY2 is a testis-specific protein that interacts with VCY2IP-1, which identified recently as a homologous protein with human microtubule-associated proteins [26].

Spermatogenesis-related genes of AZF regions are considered to be important for infertility research in various populations. Here we analyzed expression pattern of the RBMY1, CDY1 and VCY2 genes using RT-PCR approach in testicular tissues from 42 Korean azoospermic patients.

Materials and Methods

Patients

Testicular biopsies of 42 infertility men diagnosed as azoospermia were performed in accordance with the guideline of the Pusan National University Hospital.

RNA extraction from testicular tissues

Testicular tissues from each of the subjects were immediately placed at -80°C before RNA extraction. RNAs from the testicular tissue samples were extracted by the Trizol reagent (Invitrogen). Pure messenger RNAs were subtracted by PolyATtract mRNA isolation systems (Promega).

cDNA synthesis

The mRNAs were primed with oligo(dT) primer and RT reaction mixture which contained mRNA, 5× RT buffer, 2.5 mM dNTP, RNase inhibitor, AMV reverse transcriptase, and RNase-free water (Promega). RT conditions were the following: 55°C for 30 min, 99°C for 5 min and cooling at 4°C. G3PDH as positive control was amplified in order to validate the cDNA synthesis from mRNAs.

Oligonucleotide primers

Exon 11 and exon 12 of RBMY1 gene was amplified by a specific primer sense (5'-ATCATGATGGCTACGGTGACG -3', base 1224-1244) and antisense (5'-TATCTACCTCTCTC CACAAAAC-3', base 1719-1740) from NM_005058. Exon 1 and exon 2 of CDY1 gene was amplified by a specific primer sense (5'-GCCTGGTCTCTCAGGTATTTT-3', base 1705-1725) and antisense (5'-TCAAGCGATATCTCA CCACCA-3', base 2114-2134) from NM_004680. Exon 6 and exon 7 of VCY2 gene was amplified by a specific primer sense (5'-A TATTATGTGAAACTGTACCCG-3', base 542-563) and antisense (5'-GAGCCACGATGGATGTTATC-3', base 688-707) from NM_004678. G3PDH was amplified by the primers GPH-S (5'-CAAAGTTGTC ATGGATGACC-3', bases 31721-31740) and GPH-AS (5'-CCATGGAGAAGGCTG GGG-3', bases 31898-31915) from human G3PDH (GenBank accession no. AC068657).

PCR amplification

Using the synthesized cDNA, PCR amplification was performed with primer pairs for RBMY1, CDY1 and VCY2 genes. PCR amplification was carried out for each gene in 25 ul of reaction volume containing: 3 ul of synthesized cDNA, 2.5 mM of dNTP mixture, 10 pmol of each oligonucleotide primer, 10× PCR buffer (10 mM Tris±HCl (pH 8.0), 100 mM KCl, 2 mM MgCl2, 0.1 M EDTA, 1 mM DTT) and 0.3 unit of *Taq* polymerase using a thermocycler. After the initial denaturation step at 95°C for 5 minutes, DNA were amplified 35 cycles of 45 second denaturation at 95°C, for 45 second annealing at different temperatures ranging from 52-58°C and 45 second extension at 72°C, ended by elongation step at 72°C for 7 minutes and cooling at 4°C. PCR reaction products were separated on a 1.5-2% agarose gel by electrophoresis in 1× TBE (Tris-borate/EDTA) buffer. All PCR amplifications were performed repeatedly for experimental validation. The products were visualized by ethidium bromide staining followed by exposure to ultraviolet light.

Results

In order to understand expression pattern of Y chromosomal genes, RT-PCR analysis was performed using testicular samples of 42 Korean azoospermic patients and primer pairs of RBMY1, CDY1, and VCY2 genes. As shown in Fig. 1, the result of RT-PCR analysis shows different expression pattern of the Y chromosomal genes, and is also summarized in Fig. 2. The RBMY1 gene transcripts were not appeared from 14 infertile patients (no. 5, 11, 15, 16, 17, 18, 21, 22, 23, 24, 26, 27, 33, 34), indicating that 33 per-

cent of the samples showed no expression of RBMY1 gene in Korean male infertility (Fig. 1A). The CDY1 gene transcripts were not appeared from 27 patients (no. 2, 4, 5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 27, 28, 29, 32, 33, 34, 40, 42), indicating that 64 percent of the samples showed no expression of CDY1 gene in Korean male infertility (Fig. 1B). The VCY2 gene transcripts were not appeared from 11 infertile patients (no. 11, 12, 17, 18, 21, 22, 23, 24, 26, 27, 28), indicating that 26 percent of the samples showed no expression of VCY2 gene in Korean male infertility (Fig. 1C). Among azoospermic patients, 6 patients (no. 11, 19, 21, 22, 23, 42) were Sertoli cell-only syndrome. All samples of Sertoli cell-only syndrome showed no expression of CDY1 gene. Patients who had no expression of RBMY1 and VCY2 genes were also showed negative expression of the CDY1 gene in their testis tissues (Fig. 2). Overall, 15 infertile patients (no. 1, 3, 6, 7, 8, 9, 25, 30, 31, 35, 36, 37, 38, 39, 41) showed expression of RBMY1, CDY1, and VCY2 genes, and 9 infertile patients (no. 11, 17, 18, 21, 22, 23, 24, 26, 27) showed negative expression of the Y chromosomal genes, indicating that 64 percent of the infertile patients showed arrest expression in one of the tested genes.

Discussion

In the genetic view, spermatogenesis is a complex developmental process in relation to successive gene regulating system in human testicular tissues. The RBMY1, CDY1, and VCY2 genes could affect the spermatogenesis in testicular tissue. They have been focused by the frequent deletion on AZF region in male infertility patients [4,12,19, 23]. To investigate expression patterns on RBMY1, CDY1, and VCY2 genes, we performed RT-PCR amplification using Korean azoospermic patients (Fig. 1). Notably, 64% of patient samples showed no expression of the CDY1 gene. Negatively expressed RBMY1 gene was present in 33% of the patients. The VCY2 gene showed 26% of no expression

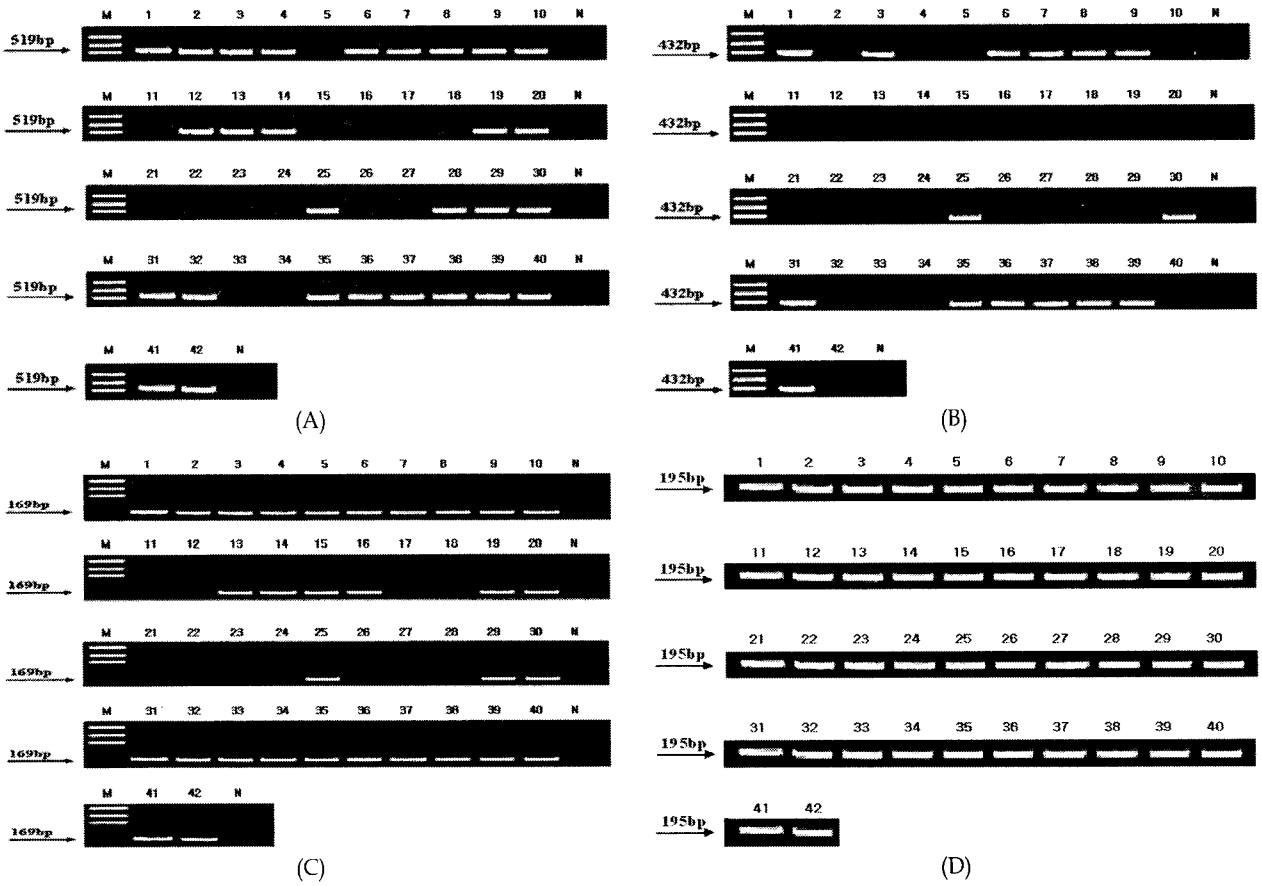

Fig. 1. RT-PCR analysis for 42 patients using primer pairs for (A) RBMY1 (519 bp), (B) CDY1 (432 bp), (C) VCY2 (169 bp), and (D) G3PDH (195 bp) genes. The numbers indicated the patients' number. M, size marker (pUC18/Taq I); N, negative control (No DNA).

Patient no.	RBMY1	CDY1	VCY2
1	■		■
2	■		■
3	■	■	■
4	■		■
5			■
6	■	■	■
7	■	■	■
8	■	■	■
9	■	■	■
10	■		■
11			
12	■		
13	■		■
14	■		■
15			■
16			■
17			
18			
19	■		■
20	■		■
21			
22			
23			
24			
25	■	■	■
26			
27			
28	■		
29	■		■
30	■	■	■
31	■	■	■
32	■		■
33			■
34			■
35	■	■	■
36	■	■	■
37	■	■	■
38	■	■	■
39	■	■	■
40	■		■
41	■	■	■
42	■		■

Fig. 2. Expression pattern of RBMY1, CDY1, and VCY2 genes in 42 Korean azoospermic patients. Solid squares indicated the positive expression and empty squares indicated the negative expression of individual genes.

patterns in tested samples. As shown in Fig. 2, infertile patients who have no expression of the RBMY1 and VCY2 genes showed no expression of the CDY1 gene in their testis tissues, suggesting that the CDY1 could be implicated to the expression of RBMY1 and VCY2 genes. These results were also correlated with previous results that the RBMY1 gene was expressed in the stage of spermatogonia, spermatocytes, and round spermatids, CDY1 gene was expressed

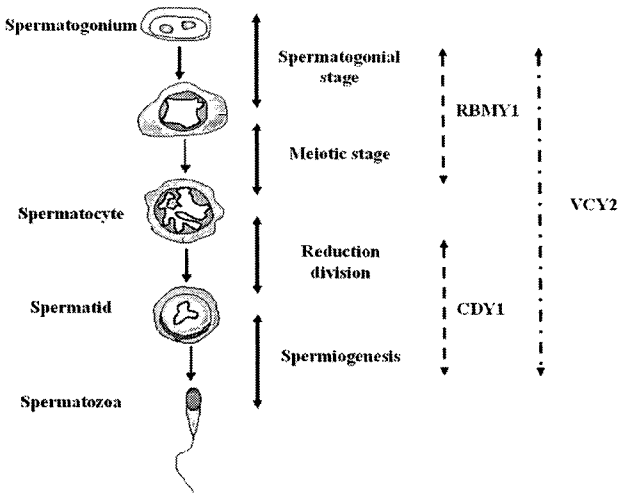

Fig. 3. Schematic representation of spermatogenesis in human testis. The RBMY1, CDY1, and VCY2 genes showed different expression patterns in different development stages (modified from Cooke and Saunders, 2002).

in the following stage spermatogenesis, and VCY2 gene in the various stages [12,13,23] (Fig 3). According to the expression data of testicular tissues in Y chromosomal genes using 42 Korean infertile men, the CDY1 gene seems to be most important factor in Korean infertile patients.

Among the testicular tissues, 6 infertile patients (no. 11, 19, 21, 22, 23, 42) were diagnosed as Sertoli cell-only syndrome. In their testis, spermatogenesis was arrested by the absence of spermatogonium. Therefore, only Sertoli cells (supporting cells) and Leydig cells (sex-hormone-secreting cells) are found in their testis. Interestingly, all tested samples of Sertoli cell-only syndrome showed no expression of CDY1 gene, suggesting that the CDY1 gene might be expressed in mainly germ cells except for Sertoli and Leydig cells during the spermatogenesis. Some of Sertoli cell-only syndrome (no. 19, 42) showed positive signals in RBMY1 and VCY2 genes (Fig. 1), indicating that the RBMY1 and VCY2 genes seem to be expressed not only in Sertoli and Leydig cells but also in spermatogenic cells.

Although RBMY1, CDY1, and VCY2 genes have influence on spermatogenesis, most problems in infertility patients are still unknown up to 50% of cases [21]. Complex unknown process (environmental factors, behavioral factors, hormone regulation, chromosome abnormality, physical problem, and genetic defects) must have been investigated to solve the mystery of spermatogenesis [5]. Many different genes on Y chromosome are specifically expressed in testis [20]. In the case of microdeletion analysis, different deletion patterns were observed in different coun-

tries [9,18]. Therefore, to solve the genetic problem of infertile men related to the AZF region genes, various genes must be investigated simultaneously based on the transcript levels. The expression study of Y chromosomal genes in testicular tissues derived from Korean infertile patients could be having great contribute to understanding of spermatogenesis.

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초록 : 한국 불임남성에 있어서 RBMY1, CDY1 그리고 VCY2 유전자의 발현 분석

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무정자증에 영향을 미치는 AZFa, b, c 영역은 남성불임환자에서 잦은 미세결실이 발견됨으로써 정자형성과정에서 중요한 역할을 할 것으로 주목 받아왔다. 이들 영역에 있는 유전자중 RBMY1, CDY1 그리고 VCY2 유전자는 고환에서 남성의 생식선 세포의 분화와 연관되어 있는 것으로 알려졌다. 42명의 무정자증 환자의 고환조직을 RT-PCR법으로 분석해본 결과 RBMY1, CDY1 그리고 VCY2 유전자는 각각 34%, 66%, 그리고 27%의 환자에서 발현되지 않는 것으로 조사되었다. RBMY1 과 VCY2 유전자가 발현되지 않는 개체는 CDY1유전자도 역시 발현이 되지 않았다. 세르토리 세포만 가진 환자에서는 CDY1 유전자가 발현되지 않았다. 따라서, CDY1 유전자는 한국인 집단에서 정자형성과정의 필수적인 요인인 것으로 사료된다.