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Differential Expression of Multiple Connexins in Rat Corpus and Cauda Epididymis at Various Postnatal Stages

Ki-Ho Lee*

Department of Biochemistry and Molecular Biology, College of Medicine, Eulji University, Daejeon, Korea 301-746

ABSTRACT

Direct cell-cell communication via the transfer of small molecules between neighboring cells in tissue is accomplished by gap junctions composed of various connexins (*Cx*s). Proper postnatal development of the epididymis is important for acquisition of male reproduction. The epididymal epithelium is composed of several cell types, and some of these cells are connected by gap junctions. The present study was conducted to determine the presence of *Cx* transcripts in the corpus and cauda epididymis. In addition, transcriptional changes of *Cx*s expressed during different postnatal stages were examined by real-time PCR analysis. In both epididymal regions, the same nine *Cx* transcripts of thirteen *Cx*s tested were detected. In the corpus epididymis, the highest levels of *Cx*s31.1 and 37 transcripts were observed at 45 days of age, and amounts of *Cx*s26, 30.3, and 32 transcripts increased with age and subsequently decreased in the elderly. Expression of *Cx*31 was greatly increased in the adult and elder stages, while *Cx*s40, 43, and 45 were abundant in the early postnatal stages. In the cauda epididymis, expression of *Cx*s26, 30.3, 31.1, 37, and 40 reached the highest levels at 5 months of age. The levels of *Cx*s31 and 32 mRNAs fluctuated throughout the postnatal period. The amounts of *Cx*s43 and 45 transcripts were more abundant during the late neonatal and prepubertal ages than later ages. These findings suggest that regional specification of the epididymis is partly regulated by differential expression of *Cx* genes during the postnatal developmental period.

(**Key words :** Connexin, Corpus epididymis, Cauda epididymis, Postnatal development, Gene expression)

INTRODUCTION

Cell-cell communication is required for the maintenance of homeostasis in mature multicellular organisms, as well as differentiation and cell proliferation during embryonic morphogenesis and postnatal maturation (Meşe et al., 2007). Communication between neighboring cells is accomplished via cell junctions. There are three types of cell junctions, adherens, gap, and tight junctions, each of which is composed of specialized proteins (Cyr, 2011). Among these, gap junctions are particularly important because they allow direct transfer of chemical messengers between adjacent cellular cytoplasm via gap junctional complexes (Cry, 2011). The complexes consist of two homo- or heteromeric hemichannels, and each hemichannel is constructed of six connexins (*Cx*s). About 20 *Cx*s have been identified in humans and rodents (Meşe et al., 2007). Almost all cells, including testicular cells, possess more than one type of *Cx*s during embryonic and postnatal development (Beyer et al., 1995; Juneja, 2003). It is important to note that not all *Cx*s can interact with each other (Segretain and Falk, 2004). Moreover, each *Cx* has unique conductance, permeability, and gating to specific molecules, indicating an increase of structural and functional diversity and complexity by *Cx*s (Goldberg et al., 2004).

Expression and physiological functions of *Cx*s in the male reproductive system have been extensively examined in a number of studies (Cry, 2011; Pointis et al., 2005 and 2010). A total of 11 *Cx*s are expressed in the seminiferous epithelium, interstitial compartment, and germ cells of mature rat testis (Pointis et al., 2005). *Cx*s in the testis is closely related to the initiation of spermatogenesis because its expression in different stages of mouse spermatogenic cells varies (Yu et al., 2003). Deletion of the *Cx*43 gene results in total depletion of germ cells and exhibits a Sertoli-cell-only feature in the testis, leading to male infertility (Plum et al., 2000). Expression of Cxs in the prostate and seminal vesicle has also been observed in several studies. The seminal vesicle expresses *Cx*32 mRNA (Meda et al., 1993), and the prostate possesses transcripts of *Cx*26, *Cx*32, and *Cx*43

* Corresponding author : Ki-Ho Lee, Ph.D. Department of Biochemistry and Molecular Biology and Medical Sciences Research Institute, Eulji University, Daejeon, S. Korea 301-746. Tel: +82-42-259-1643, Fax: +82-42-259-1649, E-mail: kiholee@eulji.ac.kr

(Pointis et al., 2005). The presence of several *Cx*s transcripts in the epididymis has been evaluated in previous studies (Cry, 2011; Han and Lee, 2013). These *Cx*s include *Cx*26, *Cx*30.3, *Cx*31, *Cx*31.1, *Cx*32, *Cx*37, *Cx*40, *Cx*43, and *Cx*45. Comparison analyses of *Cx* genes expression between the head and tail part of the epididymis from 7 to 91 days of postnatal age conducted by Dufresne et al. (2003) demonstrated segmental-specific and postnatal age-differentiated expression patterns of *Cx* genes in the epididymis. The epididymis is divided into an initial segment (IS), caput epididymis (head), corpus epididymis (body), and cauda epididymis (tail) based on morphological and functional aspects (Arrotéia et al., 2012). Because the head of the epididymis used in Dufresne's research includes the IS, caput epididymis, and corpus epididymis, it is difficult to determine whether their findings truly represent segment-specific expression of *Cx* genes. Indeed, our previous studies demonstrated that expression of *Cx* genes between the IS and caput epididymis are not identical (Han and Lee, 2013; Seo et al., 2010). Therefore, more careful examination of the expression of *Cx* genes in each epididymal segment is required to determine the actual segment-specific expression of *Cx* genes in the epididymis.

The epididymis plays several important functional roles in male reproduction, including sperm maturation and storage (Arrotéia et al., 2012). The epididymis has a single highly coiled tubular structure composed of an epithelial layer covered by a smooth muscle layer (Sullivan, 2004). The epididymal epithelium is composed of several cell types, including principal, narrow, basal, apical, halo, and clear cells (Arrotéia et al., 2012). Regional-specific distribution of the cell types in the epididymis has been determined in other studies. The principal cell is the most common type of cell present throughout the epididymis, even though the number of principal cells gradually decreases toward the epididymal tail (Arrotéia et al., 2012; Robaire and Hermo, 1988). Differentiation of the epididymal epithelial cells occurs in two separate stages during the postnatal period. The first stage is characterized by the appearance of halo cells during early infancy and the second stage is completed by development of other cell types at adulthood (Arrotéia et al., 2012). It is well known that each cell type in the epididymis plays different roles contributing to the epididymal functions. For example, principal cells secrete a majority of the epididymal proteins, while narrow cells control intracellular transport of molecules from the lumen (Robaire et al., 2006). Together, distinct cellular compositions along the epididymal duct established during postnatal development would contribute to the creation of a unique regional-specific microenvironment. Therefore, it is logical to assume that appropriate expression of *Cx* genes in different epididymal regions during the postnatal period is necessary for creation of cellular harmony among differentiated cells and therefore for foundation and maintenance of epididymal functions.

Comprehensive examination of the regional specific expression of *Cx* genes in the epididymis throughout the entire postnatal period has not been conducted. Our previous study showed differential expression of *Cx* genes in the rat epididymis from 1 week of age to 2 years of age (Han and Lee, 2013). In the present study, expressional patterns of *Cx*s in the corpus and caudal epididymis of rat were investigated by quantitative real-time PCR analyses while paying careful attention to differences in the expression of *Cx* genes among epididymal regions.

MATERIALS AND METHODS

1. Experimental animals and tissue collection

In the present study, a total of seven experimental age groups of male Sprague Dawley rats were used: early neonatal, 1 week of age (n=10); late neonatal, 15 days of age (n=10); pre-pubertal, 25 days of age (n=8); pubertal, 45 days of age (n=7); two adult stages, 5 months of age (n=5) and 1 year of age (n=3); old, 2 years of age (n=3). Young animals at 1 week of age and 15 days of age were obtained from pregnant female animals acquired from Samtako (OSan, Korea). Animals at 25 and 45 days of age were purchased directly from Samtako. Animals in the rest of the experimental groups were generously provided by the Aging Tissue Bank (Department of Pharmacology, Pusan National University, S. Korea). The present study was carried out in accordance with the guide for the care and use of laboratory animals of the National Research Council in S. Korea with the permission of university authorities.

Experimental animals were anesthetized by CO_2 stunning, after which the male reproductive tract was taken via an incision in the lower abdominal area. The epididymis was then separated from the testis and vas deferens, and the epididymal fat was trimmed away in cold PBS. The corpus and cauda epididymis were isolated from the whole epididymis under a dissecting microscope. Because of the

very small size of the corpus epididymis at 1 week of age, 15 days of age, and 25 days of age and the cauda epididymis at 1 week of age and 15 days of age, tissues of each experimental group at these ages were pooled to obtain sufficient total RNA. Tissues were then quickly frozen in liquid nitrogen and stored at −80°C until total RNA isolation.

2. Extraction of total RNA, reverse transcription reaction, and relative real-time polymerase chain reaction (PCR) analysis

The tissue was placed in a tube containing easy-Blue total RNA extraction solution (iNtRON Biotech, Sungnam, S. Korea) and homogenized with a polytron homogenizer (Fisher Scientific, Pittsburgh, PA). The RNA pellet was then extracted from the tissue by a traditional phenol-chloroform extraction method. The RNA was subsequently resuspended in RNA storage buffer (Ambion, Austin, TX) at −80°C until reverse transcription (RT) to generate the first strand cDNA. Quantitative and qualitative analyses of total RNA were conducted by UV spectrophotometry (Eppendorf, New York, NY) and gel electrophoresis, respectively.

The RT reaction was carried out in a mixture of 1 μg of total RNA with oligo-dT primer in a total volume of 20 μl

Table 1 Oligonucleotide primers for relative real-time PCR analysis

Gene (GenBank ID)	Primer sequence (5' → 3')	°C	PCR product size (bps)
*Cx*26 (NM_001004099)	(F) TCCTCTTCATCTTCCGCATC (R) CCGTTTCTTTTCGTGTCTCC	55	233
*Cx*30 (NM_053388)	(F) CAATCTCGTGGACTGCTTCA (R) ATGGCATTCTGACCGCTATG	55	243
*Cx*30.3 (NM_053984)	(F) CCCAATGTCTGCTATGACGA (R) CACAGCAGCCTTGAAGATGA	57	243
*Cx*31 (NM_019240)	(F) TTGAGCGGTGTGAACCAGTA (R) TGTTGGAGATGGGGAAGAAG	57	193
*Cx*31.1 (NM_019241)	(F) CATCGTCTGCATCCTGCTTA (R) ATGAGGTCGCTTGAGAGGAA	55	165
*Cx*32 (NM_071251)	(F) AGAATCATGGTGCTGGTGGT (R) CCTCAAGCCGTAGCATTTTC	57	235
*Cx*33 (NM_019308)	(F) TGAGAGGCAGATTGCTGCTA (R) AGACACCATTGACACCACCA	57	221
*Cx*36 (NM_019281)	(F) TCTGGAGATTGGGTTTCTGG (R) CGGACAGCCAGTTTGATCTT	58	231
*Cx*37 (NM_021654)	(F) AGTGTCTGTACCTTGGATGCC (R) CAGCACACTTAGCCAAGAGC	51	223
*Cx*40 (NM_019280)	(F) ATACCATTCAGCCTGGTTGC (R) CGGCCTCTTTAGCTTTCTCA	57	189
*Cx*43 (NM_012567)	(F) AGCAAGCTAGCGAGCAAAAC (R) GAGTTCATGTCCAGCAGCAA	55	151
*Cx*45 (NM_001085381)	(F) GATCATCCTGGTTGCTACTC (R) GATCCTCTTCATGGTCCTCT	51	173
*Cx*50 (NM_0153465)	(F) CCACTCCATTGCAGTTTCCT (R) AGAAGGCAGGGTTTCTTGGT	57	211
Ppia	(F) GGCAAATGCTGGACCAAACAC (R) TTAGAGTTGTCCACAGTCGGAGATG	59	196

Cx : connexin; *Ppia* : peptidylprolyl isomerase A (cyclophilin A).

using the ImProm-II™ reverse transcription system (Promega, Madison, WI). RT reaction conditions were as follows: 5 min at 25°C, 1 hr at 42°C, and 15 min at 70°C. The complementary DNA (cDNA) generated from the RT reactions was directly used for relative real-time PCR analysis or kept at −20°C for later use.

Relative real-time PCR was performed in a mixture of 1 μl of cDNA, 10 pmol of primer set, 10 μl of master mixture (Finnzymes, Espoo, Finland), and enough nuclease free-water to give a final volume of 20 μl. Pre-denaturation of template cDNA in the PCR mixture was first conducted at 95°C for 5 min, after which the samples were subjected to 35 cycles of denaturation at 95°C for 30 sec, annealing at T_m for 30 sec, and extension at 72°C for 30 sec, followed by final extension at 72°C for 10 min. General information regarding the oligonucleotide primers used for real-time PCR is shown in Table 1. As an internal quantitative control of the real-time PCR, cyclophilin A (*Ppia*), was also included. All PCR products were checked by fractionation in agarose gel.

3. Data collection and statistical analysis

Triplicate RT reactions and PCR from each pooled tissue were independently carried out to determine the mean and standard error (SE) of the experimental group. Triplicate RT reactions and PCR from each individual tissue were performed to obtain the mean of the experimental animal. With the exception of 1 year old and 2 year old samples, the mean and SE for the mRNA level of *Cx* expression in specific epididymal tissue at 25 days of age, 45 days of age, and 5 months of age were calculated from results of four individual tissues.

The results were presented as relative expression ratios between target *Cx* and *Ppia* at a given postnatal age. One-way ANOVA followed by a post-hoc Duncan's test was employed to determine the statistical significance among relative expression levels of *Cx* isoforms of different age groups. A P value <0.05 was considered statistically significant.

RESULTS

1. Expression profiling of *Cx* genes in the corpus and cauda epididymis and expression patterns of *Cx*26, *Cx*30.3, and *Cx*31 in the corpus epididymis during the postnatal period

Real-time PCR analysis revealed expression of 9 *Cx* isoforms of the 13 *Cx*s examined in the corpus and cauda epididymis, including *Cx*s26, 30.3, 31, 31.1, 32, 37, 40, 43, and 45. Fig. 1 shows the expression patterns of *Cx*s26, 30.3, and 31 in the corpus and cauda epididymis. In the corpus epididymis, the level of *Cx*26 transcript was significantly increased at 15 days of age and 25 days of age, then gradually decreased throughout the rest of the postnatal period (Fig. 1A). Expression of the *Cx*30.3 gene was significantly increased at 15 days of age, and the highest level of *Cx*30.3 transcript was detected at 45 days of age. There was no significant change of *Cx*30.3 expression after the pubertal stage (Fig. 1B). Similar to *Cx*26 and *Cx*30.3, expression of the *Cx*31 gene was significantly increased at 15 days of age (Fig. 1C). Another increase in *Cx*31 transcript level was detected at the adult stage of 5 months of age, and expression of the *Cx*31 gene was further increased at 2 years of age (Fig. 1C).

The expression patterns of *Cx*s26, 30.3, and 31 in the cauda epididymis were obviously different from those in the corpus epididymis. There was no change in *Cx*26 transcript level in the cauda epididymis until 45 days of age (Fig. 1D). However, expression of the *Cx*26 gene was significantly upregulated at 5 months of age and remained at the transcript level until 2 years of age (Fig. 1D). There were three increases in *Cx*30.3 gene expression in the cauda epididymis (Fig. 1E). The first increase was detected at 15 days of age, followed by a second increase at 45 days of age (Fig. 1E). The highest level of *Cx*30.3 transcript was observed at 5 months of age, while a significant decrease was observed at 1 year of age, where it remained until 2 years of age (Fig. 1E). The expression pattern of *Cx*31 in the cauda epididymis fluctuated (Fig. 1F). Specifically, a significant increase of *Cx*31 transcript level was observed at 25 days of age, but the level of *Cx*31 transcript was greatly decreased at 5 months of age (Fig. 1F). The abundance of *Cx*31 transcript at 1 year of age or 2 years of age was significantly higher than that at 5 months of age (Fig. 1F).

2. Expression patterns of *Cx*31.1, *Cx*32, and *Cx*37 in the corpus and cauda epididymis during the postnatal period

The expression of *Cx*31.1 in the corpus epididymis was significantly increased at 15 days of age, followed by another large increase in transcript abundance at 45 days of

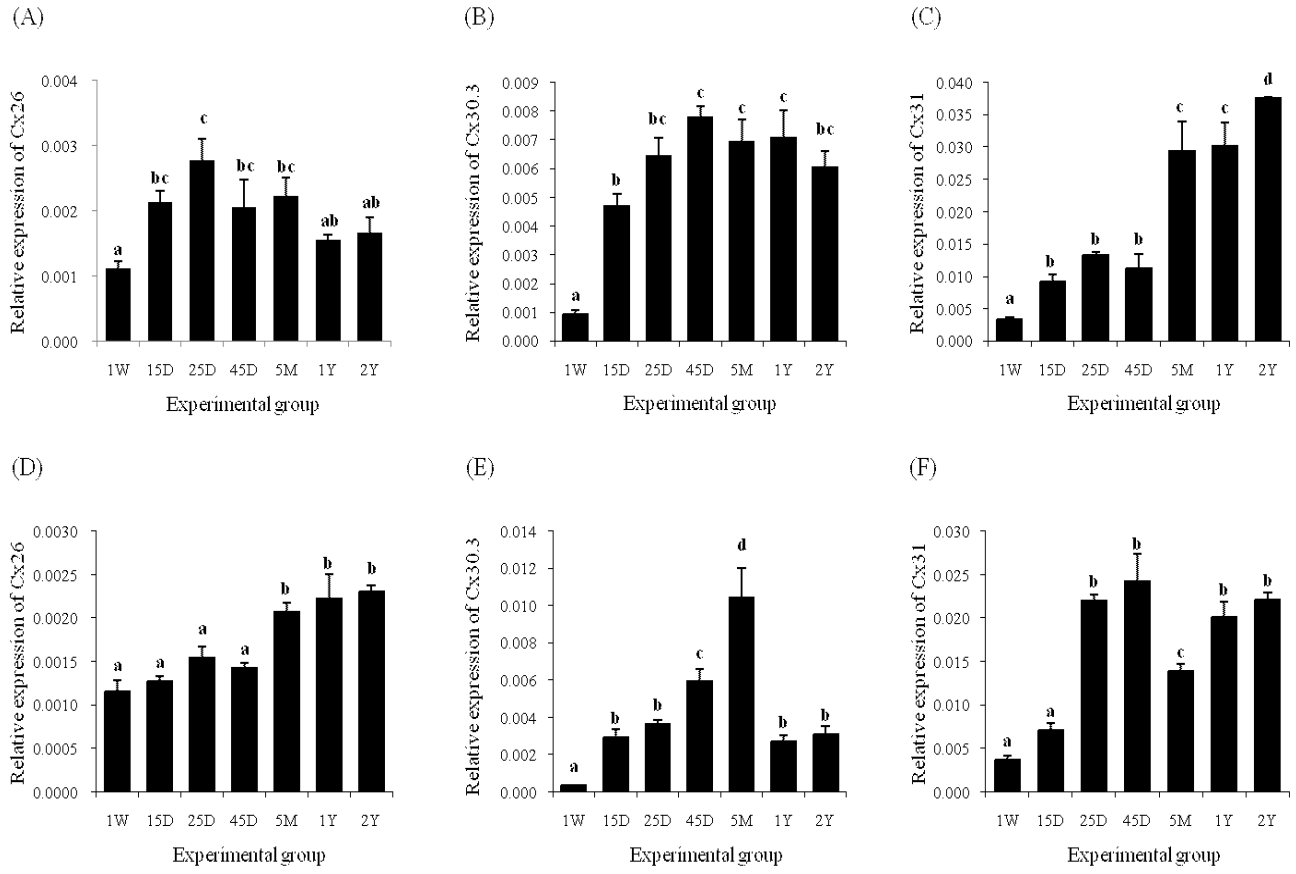

Fig. 1. Expression patterns of *Cx*26, *Cx*30.3, and *Cx*31 in the rat corpus and cauda epididymis during the postnatal period. The relative expression of *Cx*26 (A and D), *Cx*30.3 (B and E), and *Cx*31 (C and F) in the corpus (A-C) and cauda (D-F) epididymis are shown. Different letters indicate statistically significant differences among experimental groups ($P<0.05$). D: day, M: month, W: week, and Y: year.

age (Fig. 2A). However, a transient decrease of *Cx*31.1 transcript level was detected at 5 months of age, and there was no significant change in *Cx*31.1 gene expression occurred until 2 years of age (Fig. 2A). Gene expression of *Cx*32 was greatly increased at 25 days of age and reached the highest level at 5 months of age (Fig. 2B). The level of *Cx*32 transcript was gradually decreased at 1 year of age and 2 years of age (Fig. 2B). There was no change in *Cx*37 gene expression until 25 days of age (Fig. 2C) however, its expression increased significantly at 45 days of age, which was followed by a transient decreased to the levels that were observed at 1 week of age (Fig. 2C).

Expression patterns of *Cx*s31.1, 32, and 37 in the cauda epididymis differed from those in the corpus epididymis (Fig. 2D, E, and F). The abundance of the *Cx*31.1 transcript was significantly increased at 15 days of age relative to that at 1 week of age (Fig. 2D). Another great increase of *Cx*31.1 gene expression was detected at 45 days of age, followed by the highest levels being reachedat 5 months of age (Fig. 2D). The level of *Cx*31.1 transcript at 2 years of age was significantly decreased to the levels found at 45 days of age (Fig. 2D). The highest level of *Cx*32 in the cauda epididymis was observed at 15 days of age, followed by a transient decrease of *Cx*32 transcript level at 25 days of age (Fig. 2E). However, expression of the *Cx*32 gene was significantly increased at 45 days of age, after which it remained stable until 2 years of age (Fig. 2E). Expression of the *Cx*37 gene was also significantly upregulated at 15 days of age relative to that at 1 week of age (Fig. 2F). There was no change in *Cx*37 transcript level until 45 days of age (Fig. 2F). A tremendous increase of *Cx*37 gene expression was detected at 5 months of age, followed by a rapid drop at 1 year of age (Fig. 2F). An additional decrease of *Cx*37 transcript level was observed in the cauda epididymis at 2

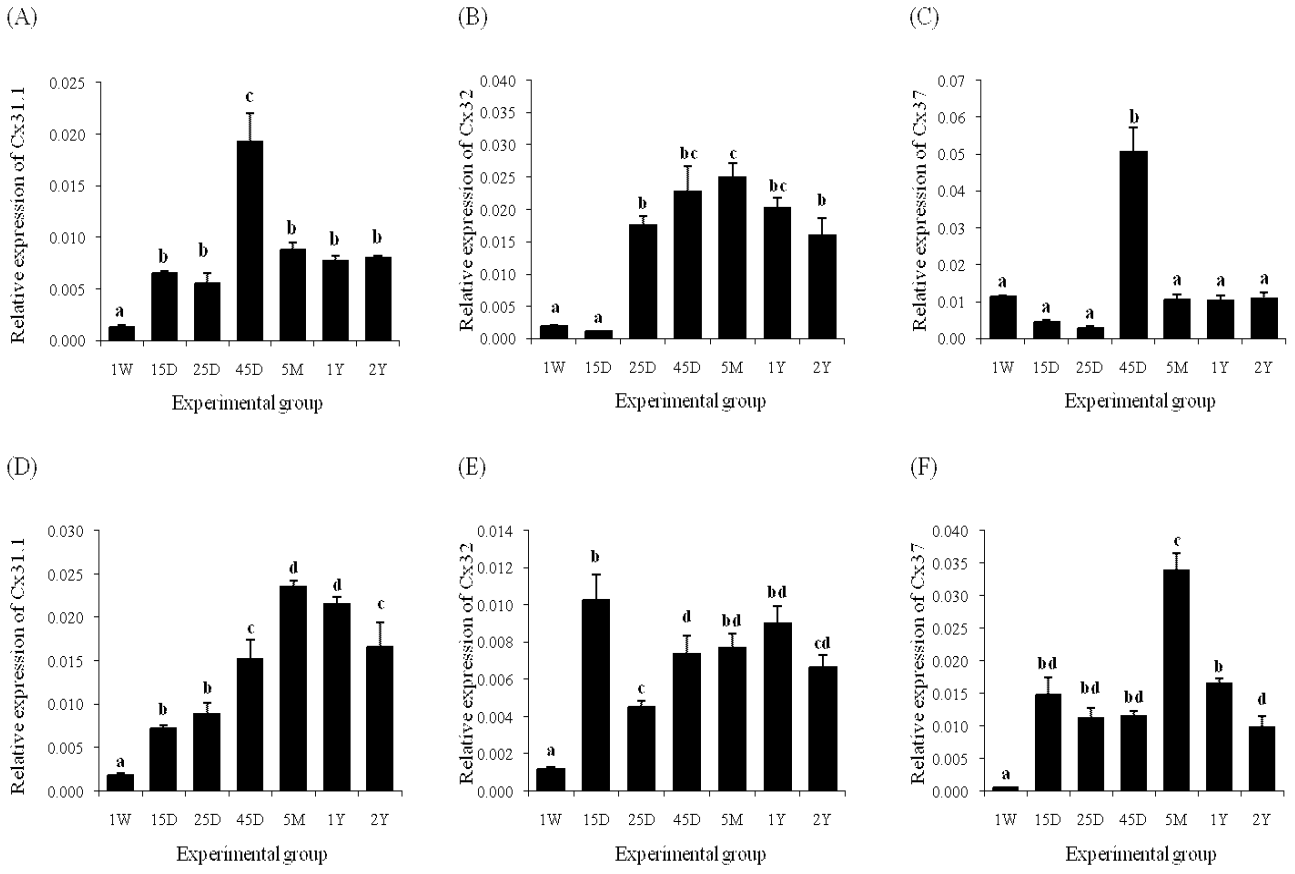

Fig. 2. Expression patterns of *Cx*31.1, *Cx*32, and *Cx*37 in the rat corpus epididymis during the postnatal period. The relative expression of *Cx*31.1 (A and D), *Cx*32 (B and E), and *Cx*37 (C and F) in the corpus (A-C) and cauda (D-F) epididymis are shown. Different letters indicate statistically significant differences among experimental groups ($P < 0.05$). D: day, M: month, W: week, and Y: year.

years of age (Fig. 2F).

3. Expression patterns of *Cx*40, *Cx*43, and *Cx*45 in the corpus and cauda epididymis during the postnatal period

Expression of *Cx*40, *Cx*43, and *Cx*45 in the corpus epididymis was particularly high during the early postnatal ages (Fig. 3). The levels of *Cx*40 transcript from 1 week of age to 25 days of age remained at a high level (Fig. 3A). However, expression of the *Cx*40 gene was drastically decreased at 45 days of age, followed by no change in *Cx*40 transcript level until 2 years of age (Fig. 3A). Similar to the expression patterns of *Cx*40 gene, high levels of *Cx*45 transcript were detected at 1 week, 15 days, and 25 days of age (Fig. 3B). However, the transcript level of *Cx*43 was significantly decreased at 45 days of age (Fig. 3B). A significant increase of *Cx*43 gene expression was observed at 5 months of age, after which they remained constant until 2 years of age (Fig. 3B). Expression of the *Cx*45 gene remained at a high level at 1 week, 15 days, and 25 days of age (Fig. 3C). The transcript level of the Cx45 gene in the corpus epididymis was significantly decreased at 45 days of age, with no change of expression occurring until 1 year of age (Fig. 3C). However, the level of the *Cx*45 transcript at 2 years of age was significantly increased to that at 25 days of age (Fig. 3C).

The expression patterns of *Cx*40, *Cx*43, and *Cx*45 in the cauda epididymis during the postnatal period are shown in Fig. 3D, 3E, and 3F, respectively. No significant change in *Cx*40 gene expression was detected until 45 days of age, while there was a remarkable increase in transcript level at 5 months of age (Fig. 3D). However, the expression level of the *Cx*40 gene at 1 year of age decreased significantly to

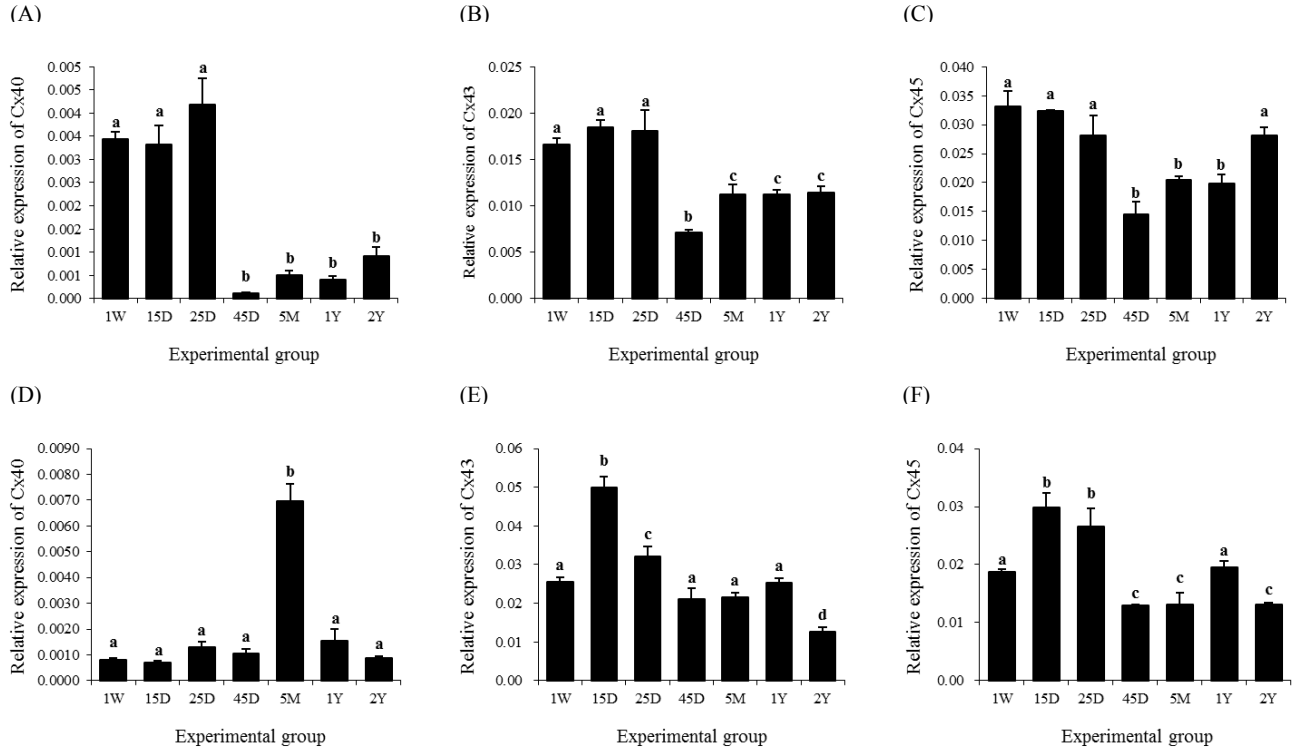

Fig. 3. Expression patterns of *Cx*40, *Cx*43, and *Cx*45 in the rat corpus epididymis during the postnatal period. The relative expression of *Cx*40 (A and D), *Cx*43 (B and E), and *Cx*45 (C and F) in the corpus (A-C) and cauda (D-F) epididymis are shown. Different letters indicate statistically significant differences among experimental groups ($P<0.05$). D: day, M: month, W: week, and Y: year.

the levels observed at 1 week of age, where they remained until 2 years of age (Fig. 3D). Expression of the *Cx*43 gene was significantly upregulated at 15 days of age relative to that at 1 week of age (Fig. 3E). However, the abundance of the *Cx*43 transcript was decreased at 25 days of age, and a further significant drop of *Cx*43 transcript level was detected at 45 days of age (Fig. 3E). An additional decrease of *Cx*43 gene expression was observed at 2 years of age (Fig. 3E). The expression of *Cx*45 fluctuated more than that of other *Cx* isoforms (Fig. 3F). The level of *Cx*45 transcript increased significantly at 15 days of age (Fig. 3F). However, a significant decrease of *Cx*45 transcript level was detected at 45 days of age (Fig. 3F). Another increase of *Cx*45 transcript level was observed at 1 year of age, but this increase was not as great as those observed at 15 days of age or 25 days of age and was followed by a decrease of *Cx*45 gene expression at 2 years of age (Fig. 3F).

DISCUSSION

The present study revealed the expression of the same types of *Cx*s in the corpus and cauda epididymis during the postnatal period, but different expressional patterns. Expression of some *Cx* genes was found to be relatively higher at neonatal and prepubertal ages than at adult and old ages, while other *Cx* genes were strongly expressed during adult and old age. Interestingly, the types of *Cx*s expressed in the caput epididymis were the same as those found in the corpus and cauda epididymis (Han and Lee, 2013). However, the expression of *Cx*26 was not observed in the IS (Seo et al., 2010), implying functional discrimination between the IS and the rest of the epididymis.

Expression patterns of some *Cx* genes in the epididymis during the first 3 months of the postnatal period were examined by Dufresne et al. (2003). Similar to our current and previous findings (Han and Lee, 2013), they reported differential expression patterns of *Cx*26, *Cx*31.1, *Cx*32, and *Cx*43 between the head of the epididymis and the cauda epididymis (Dufresne et al., 2003). However, Dufresne et al. (2003) used a mixed tissue sample of IS and caput and corpus epididymis to determine the expression of *Cx* genes in the head of the epididymis. Regional specificities of

epididymal function and physiology have been extensively demonstrated in other studies (Robaire et al., 2006). Thus, direct comparison of the findings reported by Dufresne et al. (2003) and our previous (Han and Lee, 2013) and current studies would not be appropriate. Moreover, the experimental age range differs between Dufresne et al. (2003) and that used in our study. As a result, the divergent outcomes of these investigations of the expression of *Cx* genes in the head of the epididymis are not surprising. Interestingly, comparison of the results from the present study and that conducted by Dufresne et al. (2003) revealed that the expression patterns of *Cx*31.1 and *Cx*43 in the cauda epididymis are comparable to some extent. However, due to a lack of the use of equivalent age groups, it is difficult to deliver a conclusion based on the findings of these studies. Detailed examination of individually separated segments at additional ages would provide clear evidence of the expression patterns of *Cx* genes in the epididymis for the entire postnatal period.

The cellular localization of *Cx*s in the epididymis has been observed in several studies (Cry et al., 1996; Dufresne et al., 2003). *Cx*43 is primarily localized at the base of the epididymal epithelium between principal and basal cells, as well as between basal and clear cells, suggesting coordinated epididymal functions via direct interaction with the basal cells of other cell types (Cry et al., 1996). The immunolocalization of *Cx*43 is also found in myoid cells surrounding the cauda epididymal duct (Cry et al., 1996). Additionally, the distribution of *Cx*26 and *Cx*32 in the epididymis has been reported (Dufresne et al., 2003). Adjacent principal cells possess *Cx*26 and *Cx*32, while *Cx*32 is present between apical and principal cells, between clear and principal cells, and between narrow and principal cells (Dufresne et al., 2003). These observations imply that cellular communication via *Cx*26 and *Cx*32 in the epididymis occurs through direct contact with principal cells. It is well known that each region of the epididymis is composed of distinct populations of different epithelial cell types (Arrotéia et al., 2012). For example, principal cells account for 80% of the entire epithelial population in the IS, but only 65% of those in the cauda epididymis (Robaire and Hermo, 1988). In contrast, the ratio of narrow cells increases from 3% in the IS to 6% in the corpus epididymis (Arrotéia et al., 2012). Thus, it is speculated that the segmental-specific composition of cell types would be related to differential expression patterns of *Cx* genes among different epididymal regions. Indeed, the present study revealed different expression patterns of most examined *Cx* genes between the corpus and cauda epididymis during the postnatal period. These results suggest that expressional changes of certain *Cx* genes in specific epididymal segments at certain postnatal ages contribute into the proper differentiation and maturation of the epididymis, at least in part. In addition to *Cx*s already recognized in other studies, *Cx*31, *Cx*37, *Cx*40, and *Cx*45 transcripts were identified in the corpus and cauda epididymis. Differential expression of multiple *Cx* genes in epididymal regions indicates the presence of highly coordinated cell-cell communication among different types of epithelial cells to create and maintain regional-specific functions.

Limited information is available regarding the regulation of *Cx* gene expression in the epididymis, especially in the corpus and cauda epididymis. Cry et al. (1996) showed the disappearance of *Cx*43 expression in myoid cells of the cauda epididymis in bilaterally orchidectomized rats, suggesting regulation of *Cx*43 expression by testis-derived factor (s), likely androgens. These findings are supported by those of Lydka et al. (2011), who found that treatment with anti-androgen flutamide during gestation resulted in decreased *Cx*43 expression in the cauda epididymis of boar. Chemically induced hypothyroidism results in significant decreases of *Cx*43 expression in the IS, caput and corpus epididymis, but not in the cauda epididymis, indicating regional differential regulation of *Cx*43 expression by thyroid hormone (St-Pierre et al., 2003). A recent study showed expressional regulation of *Cx*43 in the human caudal epididymis by epidermal growth factor (Dubé et al., 2012). Taken together, these findings indicate that the expression of *Cx*43 in the epididymis is clearly regulated by various hormonal factors, which are delivered from testicular and/or extra-testicular sources. The existence of androgen and EGF receptors in the rat epididymis is well documented (Tomsig and Turner, 2006). Relatively high levels of EGF receptor and EGF are found in the corpus and cauda epididymis, suggesting that EGF could influence gene expression of *Cx* molecules in these epididymal regions of the rat. The presence of estrogen and androgen receptors in the corpus and cauda epididymis has been revealed from the findings of Hess et al. (2011) and Yamashita (2004). Because serum concentrations of hormones vary during the postnatal period, particularly from the early neonatal to the pubertal ages, it is difficult to come to a conclusion regarding the expressional regulation of several *Cx* genes within the corpus and cauda epididymis.

However, the existence of complicated mechanisms associated with the expression of *Cx* genes in these tissues is obvious. Future studies are necessary to identify specific hormonal effects on theexpression of *Cx* genes in a given epididymal region at limited postnatal developmental points.

As mentioned above, the existence of various *Cx* isoforms in the corpus and cauda epididymis has been examined by other researchers. However, to the best of our knowledge, the present study is the first to show expressional profiling of *Cx* genes in these epididymal regions from the neonate to the elderly stage. We cannot exclude the possibility of expression of other *Cx* genes not being investigated in the present study. In conclusion, expression of *Cx* genes in the epididymis occurs in a regional-specific fashion, and expression patterns of *Cx* genes in the corpus and cauda epididymis are independent of each other during the postnatal developmental period.

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