Journal of Clinical Laboratory Analysis 30: 196–199 (2016)

Expression of Phosphodiesterase 4B cAMP-Specific Gene in Subjects With Cryptorchidism and Down's Syndrome

Michele Salemi,[2]* Rosita A. Condorelli,[1] Sandro La Vignera,[1] Roberto Castiglione,[1] Maria Grazia Salluzzo,[2] Carmela M. Bonaccorso,[2] Mirella Vinci,[2] Paolo Bosco,[2] Carmelo Romano,[2] Cristina Campagna,[1] Corrado Romano,[2] and Aldo E. Calogero[1]

[1]Section of Endocrinology, Andrology and Internal Medicine, Department of Medical and Pediatric Sciences, University of Catania, Catania, Italy

[2]IRCCS Oasi Institute for Research on Mental Retardation and Brain Aging, Troina, EN, Italy

Cryptorchidism represents a risk factor for infertility and germ cell testicular neoplasia. An increased rate of cryptorchidism has been reported in subjects with Down's syndrome. Cyclic nucleotide phosphodiesterases (PDEs) are important messengers that regulate and mediate a number of cellular responses to extracellular signals, such as neurotransmitters and hormones. PDE4B, cAMP-specific (PDE4B) gene which maps to chromosome 1p31.3 appears to be involved in schizophrenia, chronic psychiatric illness, learning, memory, and mood disturbances. Expression of PDE4 enzymes have been studied in testes of cryptorchid rats. Expression of PDE4B protein examination showed marked degenerative changes in the epithelial lining of the seminiferous tubules. These findings led us to evaluate PDE4 mRNA expression in leukocytes of peripheral blood of five men with DS and cryptorchidism and eleven subjects with DS without cryptorchidism compared with healthy men (controls) by quantitative Real Time PCR (qRT-PCR). This study showed that the *PDE4B* gene was downexpressed in men with DS and cryptorchidism compared to normal controls and DS without cryptorchidism. A lower expression of the *PDE4B* gene may be involved in the neurological abnormalities in subjects with Down's syndrome. Moreover, *PDE4B* gene may be involved in the testicular abnormalities of men with DS and cryptorchidism. J. Clin. Lab. Anal. 30:196–199, 2016. © 2014 Wiley Periodicals, Inc.

Key words: cryptorchidism; Down's syndrome; PDE4B gene; qRT-PCR; mRNA expression

INTRODUCTION

Cryptorchidism is the most frequent defect of the male urogenital tract at birth (1). It represents a risk factor for infertility whose rate is inversely proportional to the age when orchidopexy is carried out (1, 2). In addition, cryptorchidism increases the risk of developing germ cell testicular neoplasia by —five to ten times (3). The molecular basis of these defects seem to relate to proapoptotic pathway activation (3). An increased rate of cryptorchidism has been reported in children with Down's syndrome (DS). In a proportion of them, testes are within the scrotum at birth, but may subsequently move to an ectopic position (4). The prevalence of undescended testes in DS is 6.5% (24/368), with 4.4% (16/368) being acquired undescended or ascending testes as reported in patients with trisomy 21 who underwent surgery for undescended testes in two tertiary pediatric centers (4). These results suggest an increased prevalence of cryptorchidism in DS and, in particular, the presence of a significant proportion of acquired cryptorchidism (4). Different studies were performed on leukocytes of subjects with DS as, PCNT, KIF21A, RRP1B, and ADRB2, where there are alteration of expression of some genes in individuals with DS, compared with their respective controls (5, 6).

*Correspondence to: Dr. Michele Salemi, IRCCS Associazione Oasi Maria SS Institute for Research on Mental Retardation and Brain Aging, Troina, Italy. E-mail: micezia@tiscali.it

Received 19 May 2014; Accepted 21 November 2014
DOI 10.1002/jcla.21835
Published online in Wiley Online Library (wileyonlinelibrary.com).

Cyclic nucleotide phosphodiesterases (PDEs) are important, these proteins regulate the intracellular concentrations of the cyclic nucleotides cAMP and cGMP, and thereby play a role in signal transduction of neurotransmitters and hormones (7). *PDE4B, cAMP-specific* (*PDE4B*) gene which maps to chromosome 1p31.3 (OMIM 600127), is a class IV cAMP-specific. It appears to be involved in schizophrenia, chronic psychiatric illness, learning, memory, and mood disturbances (8).

Expression changes of PDE4 enzymes have been studied in the abdominal and scrotal testes of cryptorchid rats. Histological examination has shown marked sdegenerative changes in the epithelial lining of the seminiferous tubules. These changes included degeneration of some spermatogonia, apoptosis of the secondary spermatocytes, and incomplete spermatogenesis (9).

MATERIALS AND METHODS

The aim of this study was to evaluate PDE4B mRNA expression in leukocytes of peripheral blood of five men with DS and cryptorchidism and in six DS men without cryptorchidism in comparison with 11 healthy noncryptorchid men (controls) by quantitative Real Time PCR (qRT-PCR).

The diagnosis of trisomy 21 was performed by karyotype analysis which showed a classical chromosome 21 trisomy in all of them. The patients were referred to the Division of Andrology and Endocrinology, University of Catania for the andrological work-up. Cases and controls were recruited after family and/or personal informed consent and the ethics committee of the endocrinology, andrology, and internal medicine section gave consent to conduct the research in question.

The diagnosis of undescended testis has been made by physical examination and confirmed by ultrasound scrotal scan. The karyotype analysis of controls resulted normal. The 11 subjects with DS and the 11 age- and sex-matched controls were enrolled after family or personal informed consents were obtained in a specialized referral centre for subjects with DS. All DS subject with cryptorchidism have been reported in a previous publication (10).

The differential expression of PDE4B mRNA between men with DS and cryptorchidism and controls was evaluated by qRT-PCR in peripheral blood leukocytes. Total RNA was extracted as previously reported (10). RNA quantity and purity were confirmed by spectrophotometry and agarose gel electrophoresis. To avoid any genomic DNA contamination during qRT-PCR, a brief incubation of the samples at 42°C with a specific Wipeout buffer (QuantiTect Reverse Transcription Kit, QIAGEN Sciences, Germantown, PA) was carried out. Retrotranscription of 600 ng of total RNA from each samples was then performed in a final volume of 20 μl and generated cDNA was used as a template for real-time quantitative PCR analysis using gene expression products. For each sample qRT-PCR reactions were carried out in duplicate using 2.5 μl of cDNA and QuantiTect Probe PCR Master Mix Kit (QIAGEN Sciences, Germantown, PA) in a total volume of 50 μl. QRT-PCR experiments were performed using the ABI PRISM 7700 Sequence Detection System from Applied Biosystems. The target PDE4B gene (ID TaqMan Assay PDE4B 00963642-ml) and the reference gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) (ID TaqMan Assay GAPDH Hs99999905-ml) assays were obtained from Applied Biosystems (Carlsbad, CA, USA). The thermal cycling conditions consisted of one cycle for 2 min at 50°C, one cycle of 15 min at 95°C and 40 cycles for 15 s at 94°C followed by 1 min at 60°C. The amplified transcripts were quantified using the comparative CT method and relative quantification analysis data were played using the comparative $\Delta\Delta$Ct method. *PDE4B* gene expression level was normalized to *GAPDH* level and Target Mean Cp definition was used to indicate the mean normalized cycle threshold (10,11). Results were analyzed by one way analysis of variance (ANOVA) followed by the Duncan's multiple range test and the statistical significance was accepted when the *P*-value was lower than 0.05.

RESULTS

The five DS subjects with cryptorchidism had a mean (±SEM) age of 44.2 ± 1.16 years and the five matched controls were 43.4 ± 1.63 years old. The six DS men without cryptorchidism were 43.7 ± 0.99 years old and their six matched controls 43.2 ± 0.95 years old. No statistically significant difference resulted. DS patients with cryptorchidism had a mean total testicular volume of 19.6 ± 1.0 ml, significantly lower than that of their five matched controls (28.4 ± 1.2 ml), DS men without cryptorchidism (29.8 ± 0.89 ml), and their six matched controls (30.1 ± 1.0 ml). The *PDE4B* gene had a lower expression in men with DS and cryptorchidism compared to normal controls. Men with DS and cryptorchidism had a mean (±SEM) PDE4B expression of 0.55 ± 0.08 which resulted significantly lower compared to that of the five matched controls arbitrarily assumed to be 1 (Table 1) ($P < 0.001$). In contrast, DS subjects without cryptorchidism did not show a uniform pattern of *PDE4B* gene expression (mean ± SEM: 2.75 ± 1.93) which did not result significantly different from that of their six matched controls (Table 1).

DISCUSSION

This finding obtained in a limited number of men with DS and cryptorchidism showed about 45% lower expression of the *PDE4B* gene compared to normal men.

TABLE 1. mRNA PDE4B Gene Expression in Subjects With Down's Syndrome (DS) and Cryptorchidism (CR), DS men Without Cryptorchidism, and Normal Controls (CTL)

Sample ID	Age (years)	PDE4B (target) gene (mean Cp)	GAPDH (reference) gene (mean Cp)	Normalized ratio
CTL 1	49	26.91	24.52	1.000
DS + CR 1	48	25.74	22.87	0.783
CTL 2	43	24.15	20.79	1.000
DS + CR 2	45	22.44	17.92	0.447
CTL 3	44	26.36	33.80	1.000
DS + CR 3	44	27.20	33.51	0.458
CTL 4	42	33.02	37.01	1.000
DS + CR 4	43	32.76	34.78	0.346
CTL 5	39	24.26	20.96	1.000
DS + CR 5	41	24.74	20.92	0.695
CTL 6	45	38.22	35.36	1.000
DS 6	47	33.63	28.92	0.276
CTL 7	44	35.70	31.00	1.000
DS 7	45	33.48	20.10	1.249
CTL 8	46	33.60	33.00	1.000
DS 8	44	35.24	31.06	0.669
CTL 9	43	37.81	32.41	1.000
DS 9	44	32.78	31.00	12.32
CTL 10	40	35.94	30.86	1.000
DS 10	42	37.03	30.67	0.410
CTL 11	41	36.32	30.63	1.000
DS 11	40	37.36	32.31	1.556

Cp: crossing points.

Functional variation of *PDE4B* gene expression may impact mitochondrial cAMP catabolism with a concomitant physiological and psychiatric outcome; it may also have a role in neuronal death through a not well known mechanism (8, 12, 13). Millar and colleagues (2005) provided primary evidence for *PDE4B* gene as a genetic susceptibility factor for schizophrenia. Indeed, a link between schizophrenia and *PDE4B* may occur at the molecular level of cAMP signaling system (8). Recent studies whose purpose was to characterize PDEs suggest that cAMP-dependent regulatory pathways are indeed involved in rat spermatogenesis. Moreover, PDE4 enzyme in cryptorchidism induces apoptosis of the germ cells (14, 15). Immunocytochemical examination in rat suggested that PDE4B was predominantly expressed in the testicular somatic cells (9). This is the first study reporting the presence of a correlation between an abnormal *PDE4B* gene expression and cryptorchidism in humans. In conclusion, we found that men with DS and cryptorchidism have a lower blood leukocyte *PDE4B* gene expression compared to men with DS but without cryptorchidism. These preliminary data suggest that the *PDE4B* gene may play a role in the onset of cryptorchidism in men with DS. However, more data are needed not only in DS subjects, but also in patients with cryptorchidism alone before any more definitive conclusion may be drawn.

REFERENCES

1. Fonkalsrud EW. Current concepts in the management of the undescended testis. Surg Clin North Am 1970;50:847–852.
2. Liu Y, Li X. Molecular basis of cryptorchidism-induced infertility. Sci China Life Sci 2010;53:1274–1283.
3. Hutson JM, Balic A, Nation T, Southwell B. Cryptorchidism. Semin. Pediatr. Surg 2010;19:215–224.
4. Chew G, Hutson JM. Incidence of cryptorchidism and ascending testes in trisomy 21: a 10 year retrospective review. Pediatr Surg Int 2004;20:744–747.
5. Salemi M, Barone C, Romano C, et al. Gene expression profiling and qRT-PCR expression of RRP1B, PCNT, KIF21A and ADRB2 in leucocytes of Down's syndrome subjects. J Genet 2012;91:e18–23.
6. Li CM, Guo M, Salas M, Schupf N, et al. Cell type-specific over-expression of chromosome 21 genes in fibroblasts and fetal hearts with trisomy 21. BMC Med Genet 2006;7:24.
7. Milatovich A, Bolger G, Michaeli T, Francke U. Chromosome localizations of genes for five cAMP-specific phosphodiesterases in man and mouse. Somat Cell Mol Genet 1999;20:75–86.
8. Millar JK, Pickard BS, Mackie S. DISC1 and PDE4B are interacting genetic factors in schizophrenia that regulate cAMP signaling. Science 2005;310:1187–1191.
9. Farooqui SM, Al-Bagdadi F, Houslay MD, et al. Surgically induced cryptorchidism-related degenerative changes in spermatogonia are associated with loss of cyclic adenosine monophosphate-dependent phosphodiesterases type 4 in abdominal testes of rats. Biol Reprod 2001;64:1583–1589.
10. Salemi M, Longo GA, La Vignera S, et al. SPAG5 mRNA is over-expressed in peripheral blood leukocytes of patients

with Down's syndrome and cryptorchidism. Neurol Sci 2013;34: 549–351.
11. Livak KJ, Schmittgen TD. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)). Methods 2001;4:402–408.
12. Xu RX, Hassell AM, Vanderwall D, et al. Atomic structure of PDE4:insights into phosphodiesterase mechanism and specificity. Science 2000;288:1822–1825.
13. Szpirer C, Szpirer J, Riviere M, et al. Chromosomal localization of the human and rat genes (PDE4D and PDE4B) encoding the cAMP-specific phosphodiesterases 3 and 4. Cytogenet Cell Genet 1995;69:11–14.
14. Naro F, Zhang R, Conti M. Developmental regulation of unique adenosine 3′-5′-monophosphate-specific phosphodiesterase variants during rat spermatogenesis. Endocrinology 1996;137:2464–2472.
15. Morena AR, Boitani C, Grossi SD, et al. Stage and cell specific expression of the adenosine 3′-5′ monophosphate-phosphodiesterase genes in the rat seminiferous epithelium. Endocrinology 1995;36:687–695.