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PŮVODNÍ PRÁCE

The Association of Interleukin-6 (IL-6) -572G/C and Transforming Growth Factor Beta 1 (TGFB1) 29C/T Single Nucleotide Polymorphisms (SNPs) with Developmental Dysplasia of the Hip: a Case Control Study

Vztah interleukinu-6 (IL-6) -572G/C a transformačního růstového faktoru beta 1 (TGFB1) 29C/T jednonukleotidový polymorfismus (SNPs) s vývojovou dysplazií kyčle: řízená případová studie

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ABSTRACT

PURPOSE OF THE STUDY

The aim of the present study was to determine the potential effects of single nucleotide polymorphisms (SNPs) of TGFB1 and IL-6 on the development and severity of the disease in patients with DDH and investigate the relationship of these two gene polymorphisms.

MATERIAL AND METHODS

This case control study was conducted on 105 patients diagnosed with DDH and 119 healthy control subjects of any age. The DDH patients were classified according to the Hartofilakidis and IHDI classifications for adult and pediatric patients, respectively. Genomic DNA was isolated from peripheral blood samples using the Salting-out method. TGFB1 gene p.Pro10Leu (c.29C>T) (rs1800470) and IL-6 572G>C (rs1800796) polymorphisms were analyzed using Sanger DNA sequencing.

RESULTS

There was no statistically significant relationship of TGFB1 and IL-6 SNPs for DDH. When the rs1800470 and rs1800796 polymorphisms were compared according to family history, the homozygous mutation rate of TGFB1 gene was found to be significantly higher in patients with a positive family history than in patients with a negative family history. No significant relationship was found between rs1800796 polymorphisms and family history. TGFB1 homozygous mutation rate was determined to be statistically higher in the positive family history group than control group. No similar relationship was found between the negative family history group and the control group. No statistically significant relationship was determined between rs1800470 and rs1800796 and the severity of DDH.

CONCLUSIONS

rs1800796 and rs1800470 polymorphisms do not appear to be major responsible genetic factors for DDH. However, the determination of a correlation between a positive family history and homozygous mutation rate of the TGFB1 gene indicates that this gene may have a greater effect on DDH development.

Key words: developmental dysplasia of the hip, interleukin-6, transforming growth factor beta 1, case control study.

INTRODUCTION

Developmental dysplasia of the hip (DDH) is a congenital or developmental deformation or incompatibility of the hip joint (10). The incidence of DDH ranges from 1.5-20 per 1000 live births and is more common in females at a rate of 4–10:1 (5). In a previous study conducted in Turkey, the prevalence of untreated hip dislocation or subluxation was calculated as 5.9‰ (18). Involvement of the left hip is 2-fold more frequent than the right hip, and bilateral involvement constitutes 35% of the total DDH frequency (4). The form of DDH can range from mild acetabular dysplasia to moderate subluxation or severe hip dislocation (10). DDH is one of the most common musculoskeletal diseases in childhood, which can lead to disability if not diagnosed early or if left untreated. It remains of great importance in pediatric orthopedics practice and studies are ongoing.

Both genetic and environmental factors are known to have a role in the pathogenesis. Many risk factors such as female gender, breech presentation, first-born child, accompanying musculoskeletal conditions and high birthweight have been defined for DDH (16). Family history is also a well-known risk factor (7), and

autosomal dominant inheritance has been previously reported (3). For the past two decades, several studies have focused on genetic predisposition to DDH.

Several genes have been associated with DDH through case-control studies (7). Two of these genes are TGFB1 and IL-6. TGFB1 gene encodes transforming growth factor beta 1 (TGF-β1) cytokine, which is involved in the development of cartilage and controls cell growth, proliferation, differentiation and apoptosis (20). Interleukin-6 (IL-6) gene has a role in locomotor system development and encodes IL-6 acting as a pro-inflammatory cytokine and anti-inflammatory myokine (22). Both TGF-β1 and IL-6 are involved in bone remodeling and both have been associated with DDH or DDH-related osteoarthritis (8, 13). As a result of polymorphism studies of TGFB1 and IL6 conducted in European Caucasians, it has been suggested that there is a significant relationship between DDH-associated hip osteoarthritis and TGFB1 and IL6 (2, 11). Another study of IL6 and TGFB1 polymorphisms in a Han Chinese population reported a potential relationship between DDH and TGFB1 and IL6 gene polymorphisms (14).

The aim of the present study was to determine the potential effects of single nucleotide polymorphisms (SNPs) of TGFB1 and IL-6 on the development and severity of disease in patients with DDH and to investigate the relationship of these two gene polymorphisms with DDH.

MATERIAL AND METHODS

Approval for this case-control study (level 3) was granted by the institutional Ethics Committee (protocol number: 09.2018.578 dated: 07.09.2018). Informed consent was provided by all patients, or by the parents/legal guardian if aged <18 years. The study was conducted in accordance with the principles of the Helsinki Declaration.

The study included a total of 105 patients of any age diagnosed with DDH or DDH-related osteoarthritis (Group A) and 119 healthy control group subjects of any age with no known disorder (Group B).

A detailed physical and radiological examination was performed for all patients. The patients diagnosed with DDH were classified according to the Hartofilakidis and International Hip Dysplasia Institute classifications for adult and pediatric patients, respectively (15). The type of DDH was classified as dysplasia, subluxated or dislocated.

The demographic data and clinical features of the patient group and control group are summarized in Table 1.

Genomic DNA isolation

2 ml venous blood samples were taken into a 0.5 M Ethylenediaminetetraacetic acid (EDTA) (Sigma, USA) tube from the cases included in the study group. The blood samples were mixed with Red Blood Cell (RBC) lysis solution [155 mM Ammonium Chloride (AppliChem, Germany); 10 mM sodium bicarbonate (Merck, Germany); 0.5 mM EDTA (AppliChem, Germany)]. Then, after centrifugation (Hettich, Germany) of the samples at +4 °C at 13,000 rpm for 5 minutes, the supernatant was removed and RBC Lysis solution was again added to the pellet. Proteinase K enzyme (MBI Fermentas, Lithuania) of 20 mg / ml, 10% Sodium Dodecyl Sulfate (Merck, Germany) with a final concentration of 0.5%, and nuclease solution [10 mM Trischloride (Amresco, USA) pH: 8; 100 mM Sodium Chloride (Merck, Germany), 1 mM EDTA (AppliChem, Germany) pH: 8] with 2.5 times the leukocyte volume was added to the bottom pellet and kept at +56 °C. 200 µl NaCl was added to the samples taken from the oven (+56 °C for 3 hours) and the samples were vortexed until a homogeneous mixture was obtained. A further centrifugation at +4 °C 13000 rpm for 20 minutes was applied. The upper part of the mixture, which was divided into two phases, was taken into another Eppendorf tube and 95% alcohol was added which is double the total volume. After the DNA was made visible by turning the Eppendorf tube upside down, the DNA was precipitated by centrifuging at +4 °C 13,000 rpm for 5 minutes. After pouring away the supernatant part, 500 ml of 70% alcohol was added to the tube and centrifuged at +4 °C 13,000 rpm for 5 minutes. The alcohol was poured away at the end of the centrifugation and the tube was left to dry. Tris-EDTA (10 mM TrisHCl, 1 mM EDTA) solution was added to the tube and DNA was dissolved by standing overnight at +37 °C. The isolated DNA was stored at -20 °C. The quality and quantity of DNA was measured with spectrophotometer. OD260/OD280 with 1.6–1.8 was accepted. Otherwise isolation was repeated.

TGFB1 Gene p.Pro10Leu (c.29C>T) (rs1800470) and IL6 572G>C (rs1800796) SNP Genotyping

TGFB1 gene p.Pro10Leu (c.29C>T) (rs1800470) and IL-6 572G>C (rs1800796) polymorphisms were analyzed using Sanger DNA sequencing. Single-nucleotid

Table 1. Demographic data of patient group and control group

Characteristic	Patient Group	Control Group
number	105	119
age (min-max) *	32 (1 m–70 y)	13 (3 m–86 y)
male/female	11/94	29/90
family history (+/-)	34/71	NA
Acetabular dysplasia	43	NA
unilateral	31	NA
bilateral	12	NA
Acetabular subluxation	15	NA
unilateral	6	NA
bilateral	9	NA
Hip dislocation	47	NA
unilateral	24	NA
bilateral	23	NA

*median value NA – not available, m – month, y – year

Table 2. Comparison of TGFB1 and IL6 gene polymorphisms according to study groups

		Patient		Control		p
		n	(%)	n	(%)	
TGFB1	C/C	28	(26.7)	30	(25.2)	0.970 [a]
	C/T	38	(36.2)	44	(37.0)	
	T/T	39	(37.1)	45	(37.8)	
IL6	G/G	91	(86.7)	98	(82.4)	0.440 [b]
	G/C	12	(11.4)	20	(16.8)	
	C/C	2	(1.9)	1	(0.8)	

[a] Chi-square test, [b] Fisher test
C – cytosine, G – guanine, T – thymine

polymorphisms (SNPs) were amplified with a polymerase chain reaction using

5’ _AGGACCTCAGCTTTCCCTC_3’

5’_TCTTCTGCCAGTCACTTCCT_3’

and

5’_GCACAGAGAGCAAAGTCCTC_3’

5’_AGCTGAAGTCATGCACGAAG_3’primers for TGFB1 and IL-6, respectively.

The PCR products were purified using ExoSAP following the manufacturer’s protocol for sequencing. Sequence reactions were run on an ABI Prism 3130*xl* DNA Sequencer and analyzed using sequencing analysis software, version 5.4 (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics vn. 20 (SPSS Inc., Chicago, Ill., USA) software. Results were stated with descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum and maximum values). The Kolmogorov-Smirnov test was used for the comparison of two groups with normally distributed quantitative parameters, and the Mann-Whitney U-test and Kruskal-Wallis test for the comparison of two groups with non-normally distributed quantitative parameters. The Pearson χ^2 test and Fisher’s exact test were applied in the comparisons of qualitative data. A value of $p<0.05$ was accepted as statistically significant. In the selected sample, power was 90%, and alpha was 0.05.

RESULTS

No statistically significant relationship was determined between TGFB1 and IL-6 SNPs and DDH (Table 2).

When TGFB1 and IL-6 polymorphisms were compared according to bilateral or unilateral involvement and type of DDH, no statistically significant difference was found between the groups (Table 3, Table 4).

In the comparison of TGFB1 and IL-6 polymorphisms according to family history, the homozygous mutation rate of the TGFB1 gene was found to be significantly higher in patients with a positive family history than in patients with a negative family history. No significant relationship was determined between IL-6 polymorphisms and family history (Table 5).

No statistically significant relationship was found between DDH and TGFB1 and IL-6 gene polymorphisms when the female patient group was compared with the female control group and the male patient group compared with the male control group. (p=0.389, p=0.842, p=0.658, p=0.864).

The TGFB1 homozygous mutation rate was determined to be statistically significantly higher in the positive family history group than in the control group. No significant difference was determined between the negative family history group and the control group. No significant relationship was determined between IL-6

Table 3. Comparison of TGFB1 and IL6 gene polymorphisms according to unilateral or bilateral involvement of DDH

		Unilateral		Bilateral		p
		n	(%)	n	(%)	
TGFB1	C/C	18	(29.5)	10	(22.7)	0.737 [a]
	C/T	21	(34.4)	17	(38.6)	
	T/T	22	(36.1)	17	(38.6)	
IL6	G/G	51	(83.6)	40	(90.9)	0.464 [b]
	G/C	9	(14.8)	3	(6.8)	
	C/C	1	(1.6)	1	(2.3)	

[a] Chi-Square test, [b] Fisher test

Table 4. Comparison of TGFB1 and IL6 gene polymorphisms according to type of hip involvement

		Dysplasia		Subluxation		Dislocation		p
		n	(%)	n	(%)	n	(%)	
TGFB1	C/C	16	(37.2)	1	(6.7)	11	(23.4)	0.081[a]
	C/T	14	(32.6)	9	(60.0)	15	(31.9)	
	T/T	13	(30.2)	5	(33.3)	21	(44.7)	
IL6	G/G	37	(86.0)	14	(93.3)	40	(85.1)	0.750[b]
	G/C	6	(14.0)	1	(6.7)	5	(10.6)	
	C/C	0	(0.0)	0	(0.0)	2	(4.3)	

[a] Chi-Square test, [b] Fisher test

Table 5. Comparison of TGFB1 ve IL6 gene polymorphisms according to family history

		Family History (+)		Family History (-)		p
		n	(%)	n	(%)	
TGFB1	C/C	12	(35.3)	16	(22.5)	0.007[a]
	C/T	5	(14.7)	33	(46.5)	
	T/T	17	(50.0)	22	(31.0)	
IL6	G/G	28	(82.4)	63	(88.7)	0.190 [b]
	G/C	4	(11.8)	8	(11.3)	
	C/C	2	(5.9)	0	(0.0)	

[a] Chi-Square test [b] Fisher test
Post-Hoc p values: C/C-C/T p:0.062, C/C-T/T p:0.952, C/T-T/T p: 0.003.

Table 6. Comparison of TGFB1 gene polymorphisms between control group and patient group according to family history

		TGFB1						p [a]
		C/C		C/T		T/T		
		n	(%)	n	(%)	n	(%)	
Family history	Control	30	(25.2)	44	(37.0)	45	(37.8)	0.039
	Positive	12	(35.3)	5	(14.7)	17	(50.0)	
	Negative	16	(22.5)	33	(46.5)	22	(31.0)	

[a] Chi-square test

Table 7. Comparison of IL6 gene polymorphisms between control group and patient group according to family history

		IL6						p [a]
		GG		GC		CC		
		n	(%)	n	(%)	n	(%)	
Family history	Control	98	(82.4)	20	(16.8)	1	(0.8)	0.186
	Positive	28	(82.4)	4	(11.8)	2	(5.9)	
	Negative	63	(88.7)	8	(11.3)	0	(0.0)	

[a] Fisher test

polymorphisms and control group and patient group according to family history (Table 6 and Table 7).

No statistically significant correlation was determined between TGFB1 and IL-6 gene polymorphisms and the severity of DDH when dysplasia, subluxated and dislocated groups were compared to the control group separately (p=0.207, p=0.583).

DISCUSSION

Early diagnosis and treatment are of great importance in the prognosis of DDH. Female gender, breech presentation, first-born child and large birth size are well-known risk factors. The presence of musculoskeletal anomalies including torticollis, foot deformities and congenital knee dislocation have been thought to be associated with DDH (12). The investigation of these accompanying factors may be helpful for early diagnosis of DDH.

The number of studies on the genetic etiology of DDH has increased during the past two decades, and these have shown that genetic factors are influential in the pathogenesis. The relationship between DDH and various genes such as TGFB1, IL-6, TbX4, WISP3, PAPPA2, GDF5, HOXB9, HOXD9, ASPN, UQCC, COL1A1 and CX3CR1, has been determined in genetic association studies. These genes have also been shown to play a role in chondrogenesis and joint formation (1, 7, 9, 21). In a study of a Han Chinese population, it was suggested that TGFB1 and IL-6 have an important role in the onset and development of DDH. (14). That study supported two previous studies conducted in Croatia by Kolundžić and Čengić, both of which reported interactions of TGFB1 and IL-6 with DDH. Those two studies were conducted on populations of the same ethnicity and both studies used limited sampling, including only adult patients with severe DDH (2, 11). However, the results of the current study showed no statistically significant relationship of TGFB1 and IL-6 SNPs with DDH, which could be explained by ethnicity differences. Unlike other studies, the relationship between bilateral or unilateral disease and both gene polymorphisms was investigated in the current study but no statistically significant relationship was found. The family history of patients was also questioned in the current study and the patients were grouped as those with and without a diagnosis of DDH in first-degree relatives.

Familial predisposition was reported by Stevenson et al. and it was suggested that the genetic contribution leads to a 12-fold increased risk of DDH for first-degree relatives (19). A previous study in Turkey reported autosomal dominant inheritance in 16 members of a Turkish family (7). In the present study, when TGFB1 and IL-6 polymorphisms in the DDH group were examined according to family history, there was seen to be a significantly higher homozygous mutation rate in the TGFB1 gene in patients with a positive family history than in patients with a negative family history, which has not been previously described in the literature. No significant relationship was found between IL-6 polymorphisms and family history. It can be assumed that this difference between the two genes may show that TGFB 1 has more importance in the genetic etiology of DDH.

TGFB1 and IL-6 have been shown to have a role in the pathogenesis of hip osteoarthritis (6, 13). In the study by Kolundžić et al., there was reported to be a strong association of combined TGFB1 and IL-6 with severe adult OA secondary to DDH (11). The CC genotype of IL-6 has been associated with a decreased risk of osteoarthritis (17). In another study, it was suggested that TGFB1 and IL-6 cannot serve as an indicator of DDH severity, despite a role in the onset and development of DDH (14). The results of the present study did not show any relationship of TGFB1 and IL-6 with the severity of DDH.

One of the shortcomings of this study was the limited number of patients. In addition, the study focused only on TGFB1 and IL-6 gene polymorphisms. Further more extensive studies investigating other genes in larger patient samples will be able to provide more data. Another limitation of this study was the gender difference between the groups, as there was a higher female gender ratio in the patient group than in the control group. Although the age distributions were similar, the median age of the two groups was quite different. The effect of these differences on the results is debatable because as the disease is developmental, age does not have an effect on etiology and genetic factors and although DDH is more common in females, gender was not seen to have any effect on the genetic factors of disease in the current study results.

CONCLUSIONS

IL-6 and TGFB1 polymorphisms do not appear to be major responsible genetic factors for DDH, despite previous reports to the contrary. Nevertheless, the correlation found between positive family history and the homozygous mutation rate in the TGFB1 gene indicates that this gene may have a greater effect on DDH development.

Highlights

- The association between genes and DDH could differ according to ethnicity.
- TGFB1 gene homozygous mutation rate is higher in patients with a positive family history than in patients with a negative family history.
- Region examined in IL-6 has less importance in the genetic etiology of DDH.

References

1. Basit S, Hannan MA, Khoshhal KI. Developmental dysplasia of the hip: usefulness of next generation genomic tools for characterizing the underlying genes – a mini review. Clin Genet. 2016;90:16–20.
2. Čengić T, Trkulja V, Pavelić SK, Ratkaj I, Markova-Car E, Mikolaučić M, Kolundžić R. Association of TGFB1 29C/T and IL6 -572G/C polymorphisms with developmental hip dysplasia: a case-control study in adults with severe osteoarthritis. Int Orthop. 2015;39:793–798.
3. Ceylaner G, Ceylaner S, Ustünkan F, Inan M. [Autosomal dominant inheritance of congenital dislocation of the hip in 16 members of a family]. Acta Orthop Traumatol Turc. 2008;42:289–291.
4. Clinical practice guideline: early detection of developmental dysplasia of the hip. Committee on Quality Improvement, Subcommittee on Developmental Dysplasia of the Hip. American Academy of Pediatrics. Pediatrics. 2000;105(4Pt 1):896–905.
5. Dezateux C, Rosendahl K. Developmental dysplasia of the hip. Lancet. 2007;369:1541–1552.
6. Finnson KW, Chi Y, Bou-Gharios G, Leask A, Philip A. TGF-b signaling in cartilage homeostasis and osteoarthritis. Front Biosci (Schol Ed). 2012;4:251–268.
7. Gkiatas I, Boptsi A, Tserga D, Gelalis I, Kosmas D, Pakos E. Developmental dysplasia of the hip: a systematic literature review of the genes related with its occurrence. EFORT Open Rev. 2019;4:595–601.
8. Kwan Tat S, Padrines M, Théoleyre S, Heymann D, Fortun Y. IL-6, RANKL, TNF-alpha/IL-1: interrelations in bone resorption pathophysiology. Cytokine Growth Factor Rev. 2004;15:49–60.
9. Kenanidis E, Gkekas NK, Karasmani A, Anagnostis P, Christofilopoulos P, Tsiridis E. Genetic Predisposition to Developmental Dysplasia of the Hip. J Arthroplasty. 2020;35:291–300.
10. Kokavec M, Bialik V. Developmental dysplasia of the hip. Prevention and real incidence. Bratisl Lek Listy. 2007;108:251–254.
11. Kolundžić R, Trkulja V, Mikolaučić M, Kolundžić MJ, Pavelić SK, Pavelić K. Association of interleukin-6 and transforming growth factor-β1 gene polymorphisms with developmental hip dysplasia and severe adult hip osteoarthritis: a preliminary study. Cytokine. 2011;54:125–128.
12. Loder RT, Skopelja EN. The epidemiology and demographics of hip dysplasia. ISRN Orthop. 2011;2011:238607.
13. Malemud CJ. Anticytokine therapy for osteoarthritis: evidence to date. Drugs Aging. 2010;27:95–115.
14. Ma W, Zha Z, Chen K, Chen H, Wu Y, Ma J, Zeng S, Zhi L, Yao S. Genetic association study of common variants in TGFB1 and IL-6 with developmental dysplasia of the hip in Han Chinese population. Sci Rep. 2017;7:10287.
15. Narayanan U, Mulpuri K, Sankar WN, Clarke NM, Hosalkar H, Price CT; International Hip Dysplasia Institute. Reliability of a new radiographic classification for developmental dysplasia of the hip. J Pediatr Orthop. 2015;35:478–484.
16. Onay T, Gumustas SA, Cagirmaz T, Aydemir AN, Orak MM. Do the risk factors for developmental dysplasia of the hip differ according to gender? A look from another perspective. J Paediatr Child Health. 2019;55:168–174.
17. Pola E, Papaleo P, Pola R, Gaetani E, Tamburelli FC, Aulisa L, Logroscino CA. Interleukin-6 gene polymorphism and risk of osteoarthritis of the hip: a case-control study. Osteoarthritis Cartilage. 2005;13:1025–1028.
18. Songur M, Akel I, Karahan S, Kuzgun U, Tumer Y. Prevalence of untreated hip dislocation in Turkish children aged 6 months to 14 years. Acta Orthop Traumatol Turc. 2011;45:215–220.
19. Stevenson DA, Mineau G, Kerber RA, Viskochil DH, Schaefer C, Roach JW. Familial predisposition to developmental dysplasia of the hip. J Pediatr Orthop. 2009;29:463–466.
20. Vaughn SP, Broussard S, Hall CR, Scott A, Blanton SH, Milunsky JM, Hecht JT. Confirmation of the mapping of the Camurati-Englemann locus to 19q13. 2 and refinement to a 3.2-cM region. Genomics. 2000;66:119–121.
21. Zamborsky R, Kokavec M, Harsanyi S, Attia D, Danisovic L. Developmental dysplasia of hip: perspectives in genetic screening. Med Sci (Basel). 2019;7:59.
22. Zilberstein A, Ruggieri R, Korn JH, Revel M. Structure and expression of cDNA and genes for human interferon-beta-2, a distinct species inducible by growth-stimulatory cytokines. EMBO J. 1986;5:2529–2537.

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