Clinical Research

ENT Updates 2016;6(1):1–4
doi:10.2399/jmu.2016001002

ENT updates
www.entupdates.org

Cytokine gene polymorphisms and expression in Turkish pediatric cochlear implant patients

Elif Baysal[1], Sibel Oğuzkan Balcı[2], Fatih Çelenk[1], Merve Kahraman[2], Murat Deniz[1], Orhan Tunç[1], Cengiz Durucu[1], Semih Mumbuç[1], Muzaffer Kanlıkama[1], Sacide Pehlivan[3]

[1]*Department of Otolaryngology, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey*
[2]*Department of Medical Biology and Genetics, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey*
[3]*Department of Medical Biology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey*

Abstract

Objective: We assessed the association between the polymorphisms and expressions of three cytokine genes and clinical parameters in children who underwent cochlear implantation due to profound congenital sensorineural hearing loss.

Methods: We analyzed the IL-6/-174, IFN-γ/+874 and TNF-α/-308 genes in 64 cases with congenital sensorineural hearing loss and in 70 healthy controls. Cytokine genotyping/expression was performed using the PCR-SSP method.

Results: No significant differences were detected between the patient group and the healthy controls with respect to the distributions and numbers of genotypes and alleles of TNF-α or IL-6. However, the TT genotype, associated with high expression of IFN-γ, and the T allele frequency were significantly more frequent in the patient group versus the controls (p=0.016 and 0.023, respectively).

Conclusion: Our results suggest that high expression of the IFN-γ gene may be associated with susceptibility to the disease. Consequently, IFN-γ may be a useful marker of the etiopathogenesis of congenital sensorineural hearing loss.

Keywords: Congenital sensorineural hearing loss, cytokine gene, variation.

Özet: Sitokin geni polimorfizmleri ve Türk pediyatrik koklear implant hastalarındaki ekspresyonu

Amaç: Çalışmamızda polimorfizmler, üç adet sitokin geni ekspresyonu ve konjenital sensorinöral işitme kaybı tedavisi için koklear implantasyon uygulanmış çocukların klinik parametreleri arasındaki ilişkiyi inceledik.

Yöntem: IL-6/-174, IFN-γ/+874 ve TNF-α/-308 genleri, 64 konjenital sensorinöral işitme kaybı hastasında ve 70 sağlıklı kontrol olgusunda analiz edildi. Sitokin ekspresyonu PCR-SSP yöntemi ile gerçekleştirilmiştir.

Bulgular: Hasta grubu ve sağlıklı kontrol grubu arasında TNF-α veya IL-6'nın genotipleri ve allel frekansları sayısı ve dağılımı bakımından belirgin bir farklılık saptanmadı. Ancak yüksek IFN-γ ekspresyonu ile ilişkilendirilen TT genotipi ve T allel frekansı, hasta grubunda kontrol grubuna göre daha sıklıkla gözlendi (sırasıyla p=0.016 ve p=0.023).

Sonuç: Sonuçlarımız yüksek oranda IFN-γ geni ekspresyonunun hastalığa olan duyarlılığı arttırdığını göstermiştir. Bunu takiben IFN-γ geninin konjenital işitme kaybının etyopatogenezinde yararlı bir belirteç olabileceği sonucu ortaya çıkmıştır.

Anahtar sözcükler: Konjenital işitme kaybı, sitokin geni, varyasyon.

Congenital hearing loss is a common birth defect that affects approximately 1–3 children per 1000 births.[1] Among the etiological factors affecting prelingual deaf patients, 60% are hereditary, while 40% are environmental or iatrogenic.[1,2] The etiology of congenital sensorineural hearing loss remains largely unknown. One hypothesis is that immunological factors are responsible for disease development.[3,4] Moreover, single nucleotide polymorphisms (SNPs) at position -308 of the TNF-α gene, at position -174 of the IL-6 gene, and at position +874 of the interferon-gamma (IFN-γ) gene were previously shown to be associated with inflammatory and autoimmune disorders.[5,6]

Correspondence: Sacide Pehlivan, Professor. Department of Medical Biology, Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey.
e-mail: psacide@hotmail.com

Received: December 20, 2015; **Accepted:** February 7, 2016

The study was presented as a poster presentation at the European Society of Human Genetics Conference, June 8–11, 2013, Paris, France (Poster no: P03.24).

Online available at:
www.entupdates.org
doi:10.2399/jmu.2016001002
QR code:

deomed

TNF-α is a potent immunomodulatory and proinflammatory cytokine that mediates inflammatory diseases and is produced by activated macrophages.[7,8] TNF-α (-308) AA and AG genotypes have the potential to produce higher levels of TNF-α, whereas the GG genotype produces lower levels of TNF-α.[9] IL-6 is a proinflammatory cytokine and plays a key role in acute and chronic inflammation, affecting the endocrine and other systems, such as the central nervous and cardiovascular systems.[10] Both the GG and GC genotypes of IL 6 (-174) are associated with increased levels of IL-6, while the CC genotype leads to decreased expression of this cytokine.[11] IFN-γ has immunomodulatory, antimicrobial, antiproliferative, and antifibrotic activities and also modulates the production or activities of several cytokines.[12] The IFN-γ (+874) AA genotype is associated with lower production, TA with intermediate production, and TT with higher production of this cytokine.[13] The aim of this study was to explore any association between the polymorphisms and expression levels of the three cytokine genes and clinical parameters in children who underwent cochlear implantation because of profound congenital sensorineural hearing loss.

Materials and Methods

In total, 64 unrelated patients with suspicion of congenital sensorineural hearing loss and 70 healthy controls were included in this study. The patients had no history of perinatal infections, and tests for viral markers for hepatitis and HIV were negative. Patients with syndromic hearing loss, malformed inner ears, or multiple handicaps were excluded. The preoperative evaluation included a medical history, a physical examination, and a battery of audiological tests (otoacoustic emissions, evoked response audiometry, behavioral audiometry, and ASSR). Imaging studies of the temporal bone were also performed. All the children then underwent cochlear implantation with Med-El (MED-EL, Innsbruck, Austria) or Nucleus (Cochlear Corp., Lane Cove, New South Wales, Australia) cochlear implants. The study was approved by the University of Gaziantep Ethics Committee of Clinical Research. All parents of the children gave their informed consent for inclusion in this study. DNA was isolated from blood samples and the IL-6/-174, IFN-γ/+874, and TNF-α/-308 cytokine genes were analyzed. Cytokine genotyping/expression testing was performed using the PCR-SSP method,[14] and all data were analyzed using the de Finetti program[15] and SPSS software (ver. 14.0 for Windows; SPSS Inc., Chicago, IL, USA).

Results

Allele frequencies and genotype distributions of the IL-6, IFN-γ, and TNF-α genes in patients and controls are shown in Table 1. There were no significant differences between the patient group and healthy controls among the distribution or number of genotypes and alleles for TNF-α or IL-6. The TT genotype, associated with higher IFN-

Table 1. Genotype distribution/expression and allele frequencies of the TNF-α, IL-6 and IFN-γ genes in patients with congenital sensorineural hearing loss and in controls.

	Genotype/ allele	Patients n* (%)	Healthy control n† (%)	OR (95% CI)	p value
TNF-·· (-308)	GG§	52 (81.3)	58 (82.9)	0.89 (0.37–2.16)	0.4933
	AG‡	11 (17.2)	10 (14.3)	1.24 (0.48–3.16)	0.4115
	AA‡	1 (1.5)	2 (2.8)	0.53 (0.04–6.10)	0.5338‖
	G allele	115 (89.8)	126 (90)	1.01 (0.45–2.25)	0.4831
	A allele	13 (10.2)	14 (10)	0.98 (0.44–2.17)	0.4831
IFN-Á (+874)	TT‡	15 (23.4)	6 (8.6)	3.26 (1.180–9.033)	0.0162‖
	TA¶	31 (48.5)	37 (52.9)	0.83 (0.42–1.65)	0.3676
	AA§	18 (28.1)	27 (38.5)	0.62 (0.30–1.29)	0.1366
	T allele	61 (47.7)	49 (35)	1.69 (1.03–2.76)	0.0239‖
	A allele	67 (52.3)	91 (65)	0.59 (0.36–0.96)	0.0239
IL-6 (-174)	GG‡	29 (45.3)	41 (58.6)	0.58 (0.29–1.16)	0.0866
	GC‡	30 (46.9)	25 (35.7)	1.58 (0.79–3.17)	0.1280
	CC§	5 (7.8)	4 (5.7)	1.39 (0.35–5.45)	0.4433‖
	G allele	88 (68.8)	107 (76.4)	0.67 (0.39–1.16)	0.1015
	C allelle	40 (31.2)	33 (23.6)	1.47 (0.85–2.53)	0.1015

*n=64, †n=70, ‡high expression, §low expression, ‖Fisher's exact Test, ¶intermediate expression.

γ expression, and the T allele frequency were found to be significantly more frequent in the patient group than in the controls (p=0.016 and 0.023, respectively). The observed genotype counts did not deviate significantly from those expected according to Hardy-Weinberg equilibrium (p>0.05). No relationship was found between the cytokine genotypes and clinical parameters of the patients.

Discussion

IFN-γ or type II interferon is a cytokine vital for innate and adaptive immunity against viral infections and intracellular bacterial infections and for tumor control. Aberrant IFN-γ expression is associated with numerous autoinflammatory and autoimmune diseases.[6] The significance of IFN-γ in the immune system stems partly from its ability to directly inhibit viral replication. Most importantly, its immunostimulatory and immunomodulatory effects are mediated predominantly by natural killer (NK) and natural killer T (NKT) cells as part of the innate immune response (and by CD4 and CD8 cytotoxic T lymphocytes (CTLs), which are effector T cells, once antigen-specific immunity develops). A functional SNP at position +874 of the human IFN-γ gene, correlating with differential cytokine production, was reported by Pravica et al.[6,14,16] The polymorphism lies within a binding site for the transcription factor NF-κB, and electrophoretic mobility shift assays showed specific binding of NF-κB to the allelic sequence containing the +874T allele. As this transcription factor induces IFN-γ expression, the +874T and +874A alleles likely correlate with high and low IFN-γ expression, respectively. The transcription factor NF-κB also preferentially binds to the +874T allele, which was overrepresented in the controls, suggesting that genetically determined variability in IFN-γ expression may be important for the development of tuberculosis.[16–19] Neutralizing IFN-γ completely prevented both vascular dysfunction and changes in NOS expression, and neutralizing TNF reduced IFN-γ production and partially prevented dysfunction. Inhibiting iNOS partially preserved responses to NO at two weeks and reduced graft intimal expansion after four weeks in vivo. In fact, Koh et al. concluded that IFN-γ was a central mediator of vascular dysfunction through the dysregulation of NO production. Adhesion development was also found to depend on the IFN-γ and STAT1 system, and NKT cell-deficient mice developed poor adhesion. However, these mice developed severe adhesion after reconstitution with NKT cells from wild-type mice, suggesting that the production of IFN-γ by NKT cells is indispensable for adhesion formation.[20] In a study by Alper et al., it is suggested that IFN-γ gene polymorphism predisposes a patient to otitis during an upper respiratory infection.[5] Matkovic et al. studied the cytokine levels of bilateral ears in patients with otitis media with effusion and they found that the immune response of the same patient can be different in each ear.[21] Aminpour et al. studied the role of TNF-α in patients with sensorineural hearing loss after bacterial meningitis and they concluded that TNF-α plays an important role in cochlear injury after bacterial meningitis.[22] According to these findings, if there is an intrauterine infection and inflammation even the immune response of the same patient might be different and this immune response can result with susceptibility for cochlear injury. According to the results of this study, silent infections in the perinatal period may contribute to congenital profound sensorineural hearing loss. To the best of our knowledge, the relationship between cytokine genotypes/expression and clinical parameters in patients with congenital hearing loss has not been investigated. Our results suggest that high expression of the IFN-γ gene may be associated with susceptibility to the disease.

Conclusion

Consequently, IFN-γ may be a useful marker for disease etiopathogenesis in patients with congenital sensorineural hearing loss especially in patients who had perinatal silent infections. There are major limitations in this study; one is the limited number of the patients with hearing loss and the second one is the markers of major viral markers were not ruled out, this was because of the limited budget. Our results allow us to make only preliminary conclusions due to the small sample size. Further studies designed with eliminating major viral infectious diseases and with larger number of patients are needed.

Conflict of Interest: No conflicts declared.

References

1. Vivero RJ, Fan K, Angeli S, Balkany TJ, Liu XZ. Cochlear implantation in common forms of genetic deafness. Int J Pediatr Otorhinolaryngol 2010;74:1107–11.
2. Lalwani AK, Castelein CM. Cracking the auditory genetic code: nonsyndromic hereditary hearing impairment. Am J Otol 1999; 20:115–32.
3. Pati S, Pinninti S, Novak Z, et al.; NIDCD CHIMES Study Investigators. Genotypic diversity and mixed infection in newborn disease and hearing loss in congenital cytomegalovirus infection. Pediatr Infect Dis J 2013;32:1050–4.
4. Lindberg E, Andersson B, Eggertsen R, Nyström E, Magnusson Y. A polymorphism at position +874 in the IFN-γ gene is associated with susceptibility for dilated cardiomyopathy. J Clin Cell Immunol 2010;1:101.

5. Alper CM, Winther B, Hendly JO, Doyle WJ. Cytokine polymorphisms predict the frequency of otitis media as a complication of rhinovirus and RSV infections in children. Eur Arch Otorhinolaryngol 2009;266:199–205.
6. Online Mendelian Inheritance in Man. Interferon, Gamma; IFNG [Internet]. Baltimore, MD: Johns Hopkins University [cited 2013 July 25] Available from: http://omim.org/entry/147570.
7. Gaur U, Aggarwal B. Regulation of proliferation, survival and apoptosis by members of the TNF superfamily. Biochem Pharmacol 2003;66:403–8.
8. Vadlamani L, Iyengar S. Tumour necrosis factor α polymorphism in heart failure/cardiomyopathy. Congest Heart Fail 2004;10:289–92.
9. Wilson AG, Symons JA, McDowel TL, McDevit HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci U S A 1997;94:3195–9.
10. Fonseca JE, Santos MJ, Canhao H, Choy E. Interleukin-6 as a key player in systemic inflammation and joint destruction. Autoimmun Rev 2009;8:538–42.
11. Fishman D, Faulds G, Jeffery R, et al. The effect of novel polymorphism in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic onset juvenile chronic arthritis. J Clin Invest 1998;102:1369–76.
12. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-gamma: an overview of signals, mechanisms and functions. J Leukoc Biol 2004;75:163–89.
13. Pravica V, Asderakis A, Perry C, et al. In vitro production of IFN-γ correlates with CA repeat polymorphisms in the human IFN-γ gene. Eur J Immunogenet 1999;26:1–3.
14. Col-Araz N, Pehlivan S, Baspinar O, Oguzkan Balci S, Severe T, Balat A. Role of cytokine gene (IFN-γ, TNF-α, TGF-β1, IL-6, and IL-10) polymorphisms in pathogenesis of acute rheumatic fever in Turkish children. Eur J Pediatr 2012;171:1103–8.
15. Rossi C. Bruno de Finetti: the mathematician, the statistician, the economist, the forerunner. Stat Med 2001;20:3651-66.
16. Ben Selma W, Harizi H, Bougmiza I, et al. Interferon gamma +874 T/A polymorphism is associated with susceptibility to active pulmonary tuberculosis development in Tunisian patients. DNA Cell Biol 2011;30:379–87.
17. Chong WP, Ip WK, Tso GH, et al. The interferon gamma gene polymorphism +874 T/A is associated with severe acute respiratory syndrome. BMC Infect Dis 2006;4:82–9.
18. Tangwattanachuleeporn M, Sodsai P, Avihingsanon Y, et al. Association of interferon gamma gene polymorphism (+874 T/A) with arthritis manifestation in SLE. Clin Rheumatol 2007: 26;1921–4.
19. Whiteman SC, Spiteri MA. IFN-gamma regulation of ICAM-I receptors in bronchial epithelial cells: soluble ICAM-I release inhibits human rhinovirus infection. J Inflamm 2008;5:5–8.
20. Doyle WJ, Casselbrant ML, Li-Korothy HS, et al. The IL-6 (-174, C/C) genotype predicts greater rhinovirus illness. J Infect Dis 2010;101:199–206.
21. Matkovic S, Vojvodic D, Baljosevic I. Comparison of cytokine levels in bilateral ear effusions in patients with otitis media secretoria. Otolarngol Head Neck Surg 2007;137:450–3.
22. Aminpour S, Tinling SP, Brodie HA. Role of tumor necrosis factor-· in sensorineural hearing loss after bacterial meningitis. Otol Neurotol 2005;26:602–9.

Please cite this article as: Baysal E, Oğuzkan Balcı S, Çelenk F, Kahraman M, Deniz M, Tunç O, Durucu C, Mumbuç S, Kanlıkama M, Pehlivan S. Cytokine gene polymorphisms and expression in Turkish pediatric cochlear implant patients. ENT Updates 2016;6(1):1–4.