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Association of PAX3 and TMTC2 genes polymorphism with the face morphology changes after excision of skin tumors

Povezanost polimorfizma PAX3 i TMTC2 gena sa promenama morfologije lica nakon ekscizije tumora kože

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Abstract

Background/Aim. The group of genes, known as PAX (paired box), has a great role in organogenesis, as well as in maintaining the normal function of certain cells after the birth. In addition to these genes, the impact on the organogenesis, at the cellular level, has a transmembrane tetratricopeptid group of genes (TMTC). The term polymorphism in the human genome implies variations in the hereditary basis that occur in human populations, the presence of two or more different alleles of one genome in the population. The aim of the work was to determine whether there is an association of PAX3 and TMTC2 genes polymorphism with changes of the face morphology after skin tumor excision and direct suture closure. **Methods.** The study included 130 patients of both sexes, older than 50 years, with the medical indication for the elliptical surgical excision of the skin tumor. DNA was isolated from 5 mL of peripheral blood. Gene polymorphisms were analyzed with pre-designed single nucleotide polymorphisms (SNP) assays, by allelic discrimination method on REAL-TIME apparatus. The patients were subjected to a laser scanning preoperatively, and 7 and 90 days postoperatively, in order to obtain x, y and z coordinates of 5 cephalometric points on the face, which determined the shape of the medial cheek region. The shape of the medial cheek region, as well as the coordinates of 5 cepahlometric points, were compared among genotypes of both genes preoperatively, as well as 7 days and 90 days postoperatively. **Results.** A statistically significant difference in the shape of the medial cheek region between wild-type and mutant of PAX3 gene was found preoperatively, while the statistically significant difference in the shape of the medial cheek region was not found between wild-type and heterozygote, nor between wild-type and heterozygote and mutant of PAX3 gene, nor among genotypes of TMTC2 gene. Seven days and 90 days postoperatively, there were no statistically significant differences in the shape of the examined region among genotypes of both genes. **Conclusion.** Polymorphisms of PAX3 and TMTC2 genes are not associated with the change in the face morphology after the skin tumor excision and direct suture closure of the defect.

Key words:
polymorphism, genetic; face; skin neoplasms; surgical procedures, operative.

Apstrakt

Uvod/Cilj. Grupa gena, poznata pod nazivom PAX (eng. *paired box*), ima velikog udela u organogenezi, kao i u održavanju normalne funkcije izvesnih ćelija nakon rođenja. Pored ovih gena, uticaj na organogenezu, na ćelijskom nivou, ima i transmembransko-tetratrikopeptidna grupa gena (TMTC). Pod pojmom polimorfizam u genomu čoveka podrazumevaju se varijacije u naslednoj osnovi koje se javljaju u humanim populacijama, prisutnost dva ili više različitih alela jednog gena u populaciji. Cilj rada bio je da se utvrdi da li postoji povezanost polimorfizma PAX3 i TMTC2 gena sa promenom morfologije lica nakon ekscizije tumora kože i postekscizione direktne suture. **Metode.** Istraživanjem je bilo obuhvaćeno 130 ispitanika, oba pola, starijih od 50 godina, kod kojih je postavljena medicinska indikacija za hiruršku elipsastu ekscisiju tumora kože lica. DNK je izolovana iz 5mL periferne krvi. Polimorfizmi gena analizirani su predizajniranim *single nucleotide polymorphisms* (SNP) esejima, metodom alelske diskriminacije na REAL-TIME aparatu. Ispitanici su skenirani laser skenerom preoperativno, kao i sedam dana i 90 dana postoperativno, kako bi se za svakog ispitanika dobile x, y i z koordinate pet kefalometrijskih tačaka na licu, koje su određivale oblik medijalne obrazne regije. Upo-

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ređivan je oblik medijalne obrazne regije, kao i koordinate pet kefalometrijskih tačaka između genotipova oba gena, preoperativno, kao i 7 i 90 dana postoperativno. **Rezultati.** Preoperativno je nađena statistički visoko značajna razlika u obliku medijalne obrazne regije između *wild type* i mutanata PAX3 gena, dok statistički značajna razlika u obliku ispitivane regije nije nađena između *wild type* i heterozigota, kao ni između *wild type* u odnosu na heterozigote i mutante PAX3 gena, kao ni između genotipova TMTC2 gena. Sedam i 90 dana postoperativno, nije nađena statistički značajna razlika u obliku ispitivane regije između genotipova, kod oba gena. **Zaključak.** Polimorfizmi PAX3 i TMTC2 gena nisu povezani sa promenom morfologije lica nakon ekscizije tumora kože lica i zatvaranja defekta direktnom suturom.

Ključne reči:
geni, polimorfizam; lice; koža, neoplazme; hirurgija, operativne procedure.

Introduction

In recent years, efforts have been intensified to determine an influence of polymorphism of genes on morphological characteristics of the face.

It has been demonstrated that a group of genes, known as PAX (Paired box), has a great role in organogenesis, as well as in maintaining the normal function of certain cells after the birth. There are four groups of PAX genes [1]. In the first group are PAX 1 and 9, in the second group are PAX 2, 5 and 8, in the third group are PAX 3 and 7, while in the fourth group are PAX 4 and 6. During embryonic development, PAX 3 gene is active in cells of the neural crest. These cells migrate from the spinal cord in certain regions in the embryo [2]. The protein encoded by PAX 3 gene influences the activity of other genes, inducing cells to form neural crest limb muscles, bones of the face and scalp, certain neural structures and melanocytes that determine the color of hair, eyes, and skin. Melanocytes are also found in some regions of the brain and the inner ear. Therefore, PAX 3 gene, associated with the development of the ear, eye and face, is highly expressed in melanoma, and also contributes to the survival of tumor cells (alveolar rhabdomyosarcoma, which is more common in adolescents). It is located on the second chromosome (2q36.1). Mutations in the gene lead to the Waardenburg syndrome. The disease is characterized by varying degrees of deafness, minor defects in structures that originate from the neural crest and anomalies in pigmentation [3].

In addition to these genes, a transmembrane tetratricopeptid group of genes (TMTC 1, 2, 3, 4) has the impact on the organogenesis, of which TMTC 2 gene encodes protein 2, which is a transmembrane building element of the cell membrane, and endoplasmic reticulum. TMTC 2 gene is located on chromosome 12 (12q21.31) [4]. At the molecular level, it has a role in binding of one molecule to one or more specific sites of other molecules [5]. The specific role of TMTC 2 gene has not yet been established, although it is known that it has a role in cellular calcium homeostasis [6].

Deoxyribonucleic acid (DNA) polymorphisms are now widely studied as markers of possible genetic susceptibility for certain diseases. The Genome-Wide Association Studies (GWAS) explain the genetic basis of complex diseases by comparing the frequency of different genetic variants in the population in relation to healthy-population. One of the most common types of genetic polymorphisms is the polymorphism of the single nucleotide polymorphism sequence (SNP), replacing one of the four nucleotides in DNA molecule. Substitutions may occur in the coding (exon) or non-coding (intron) portion of the gene, or in the promoter region. SNPs are commonly used in genetic studies of the association. Previous research has shown that SNPs can be associated with the development of various types of disease, response to pathogens, drugs and other agents. Besides, in some studies, an association between SNP of PAX3 (rs7559271, G/A) and TMTC2 (rs10862567, T/A) and differences of face morphology were found [1], but there were no studies about the association between PAX3 and TMTC2 SNP and postoperatively differences in face morphology, after skin tumor excision.

In accordance with the reconstructive ladder, in plastic and reconstructive surgery after facial skin tumor excision, we primarily use the direct closure, as this is the simplest method of covering defects [7].

However, in addition to general medical and surgical principles, it is necessary to take into account the aesthetics of the face, and the consequential symmetry after excision. If the symmetry is violated, direct suture does not apply, and we use skin graft or flap [8].

The aim of the study was to determine the association of polymorphisms of PAX3 (rs7559271, G/A) and TMTC2 (rs10862567, T/A) genes with changes of face morphology after skin tumor excision and direct suture closure of the defect.

Methods

The study included 130 patients of both sexes, older than 50 years, with the medical indication for the surgical elliptical excision of facial region skin tumors.

Before the surgical elliptical excision, 5 mL of peripheral blood of all the patients was taken by venipuncture. All the patients signed the consent to participate in the research, by the decision of the Ethics Committee of the Military Medical Academy in Belgrade. Peripheral blood with anticoagulant was kept in a freezer at -20°C. DNA from peripheral blood was isolated by commercial kit PureLink®Genomic DNA Kit (Invitrogen, Thermo Fisher, USA), according to manufacturer instructions.

Polymorphism of genes was determined with predesigned SNP (single nucleotide polymorphism) assays (TaqMan® Pre-designed SNP Genotyping Assay, Applied Biosystems, for PAX3 rs7559271, and TMTC2 rs10862567), by allelic discrimination method on REAL-TIME apparatus (ABI Prism 7500, USA).

Immediately before the surgery, in all patients, the elliptical excision of skin tumors lines around the margin of clinically unaffected skin, 2 mm width, was marked and the elliptical excision, parallel to the lines of minimum tension, was done, after which patients were scanned preoperatively with laser scanner (Laserscanner, the Institute for Robotics and Process Control, University of Braunschweig, Germany, 2009). The patients were also scanned postoperatively, 7 and 90 days after the surgery [9].

Five cephalometric points (nasion, endocanthal central point, pronazale, lower palpebral point, endocanthion), and their x, y and z coordinates, were determined from scans of the patients' face, using extraction of coordinates by C++ software, and using the characteristics of cephalometric points: nasion is the most anterior point of the junction of the nasal and frontal bones in the midsagittal plane, endocanthal central point is in the middle between bilateral most deep points of endocanthus, pronasale is the most prominent point on tip of nose, lower palpebral point is the lowest point of lower eyelid, endocanthion is the most deep point of endocanthus. For five cephalometric points we got 15 coordinates (x1-5, y1-5, z1-5). Those coordinates determined the shape of the polygonal line, as a border of the space of operated region, in the region of the medial cheek of the face. Changes of the shape of the operated region were assessed by using Procrustes analysis. As the first, superimposition of landmark points was done by translation, rotation and scaling, after what we used Procrustes distance (Pd), given by Procrustes coordinates (x, y, z), as a squared root of sum of squared distances between corresponding landmarks of two shapes, which is a measure of shape difference between two groups of shapes. We compared Pd, as a measure of shape differences, among all the genotypes, and among all three scanning times (preoperatively, 7 and 90 days postoperatively), using ANOVA and *Post Hoc* Scheffe test. Besides, as we wanted to know which coordinate has influence on changing the shape of the operated region, we compared x, y and z coordinates of five cephalometric points among the groups of patients with different genotypes of PAX3 and TMTC2 genes, using MANOVA. Determination of Pd distances was done in the software program MorphoJ, version 1.06d, 2014, while ANOVA, *Post Hoc* Scheffe test, and MANOVA, were done in SPSS 23, IBM, 2015 [10].

Results

Distribution of genotypes of PAX3 and TMTC2 gene was presented in Table 1. The most presented genotype was wild-type, in both genes.

The values of Pd means between the coordinates of each patient and average coordinates in all of three genotypes of PAX3 gene [wild-type (G/G), heterozygote (G/A), and mutant (A/A)], preoperatively, and 7 and 90 days after surgery, was presented in Figure 1. We found the highest value of Procrustes distances in all of three genotypes seven days postoperatively, while 90 days postoperatively values of Pd were lower than preoperatively. The preoperative median was lower than mean in all of the genotypes, while the median was higher than mean 7 and 90 days postoperatively, with the exception of mutants 90 days postoperatively.

Table 1
Distribution of genotypes of PAX3 and TMTC2 genes in the examined groups of patients

Genotypes	PAX3	TMTC2
	n (%)	n (%)
Wild type	72 (55.4)	103 (79.2)
Heterozygote	34 (26.1)	17 (13.1)
Mutant	24 (18.5)	10 (7.7)
Total	130 (100)	130 (100)

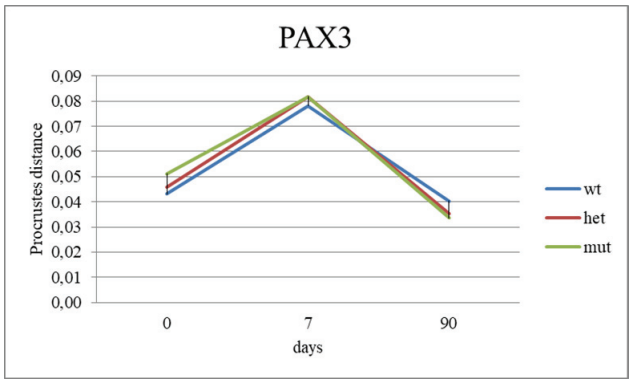

Fig. 1 – Procrustes distances in patients with different PAX3 genotypes [preoperatively (0), 7 and 90 days postoperatively].
***wt – wild type; het – heterozygote; mut – mutant.**

The statistical significance of Pd differences among the patients with different PAX3 genotypes in all of three scanning times was analyzed using ANOVA and *Post Hoc* Scheffe test and was presented in Table 2. We found the statistically highly significant difference only between wild-type and mutant preoperatively, while 7 and 90 days postoperatively there was no statistically significant difference among the genotypes.

Table 2
Significance of differences of Procrustes distances in patients with different PAX3 genotypes [preoperatively (0), 7 and 90 days postoperatively]

Genotypes	Days		
	0	7	90
	p-value		
wt vs. het	0.419	0.847	0.248
wt vs. het + mut	0.738	0.924	0.597
wt vs. mut	**0.005**	0.868	0.128

wt – wild type; het – heterozygote; mut – mutant.
***p*-values < 0.01 are bolded.**

Results of difference testing among the coordinates of PAX3 genotypes in all three scanning times (0, 7, 90 days) by MANOVA were presented in Table 3.

Table 3
Significance of differences between x, y and z coordinates in patients with different PAX3 genotypes [preoperatively (0), 7 and 90 days postoperatively]

Coordinates	Genotypes								
	wt vs. het			wt vs. het + mut			wt vs. mut		
	days								
	0	7	90	0	7	90	0	7	90
	p-value								
x1	0.292	0.292	0.292	0.934	0.901	0.874	0.677	0.677	0.677
x2	0.477	0.399	0.227	0.981	0.837	0.701	0.537	0.856	0.856
x3	0.207	0.207	0.237	0.802	0.861	0.834	0.644	0.644	0.642
x4	0.292	0.292	0.292	0.921	0.912	0.894	0.677	0.677	0.677
x5	0.292	0.292	0.292	0.934	0.911	0.901	0.677	0.677	0.677
y1	0.292	0.292	0.292	0.901	0.909	0.902	0.677	0.677	0.677
y2	0.292	0.992	1.000	**0.005**	0.961	0.574	**0.007**	0.885	0.449
y3	0.301	0.298	0.301	*0.015*	0.318	*0.019*	**0.000**	*0.016*	**0.000**
y4	0.292	1.000	0.992	**0.002**	0.981	0.521	**0.007**	0.978	0.574
y5	0.292	0.292	0.292	0.913	0.921	0.901	0.677	0.677	0.677
z1	0.590	0.281	0.281	0.958	0.825	0.827	0.706	0.723	0.723
z2	0.301	0.169	0.301	*0.016*	0.134	*0.015*	**0.000**	**0.001**	**0.000**
z3	0.572	0.508	0.508	0.928	0.922	0.924	0.476	0.861	0.861
z4	0.301	0.169	0.301	*0.017*	0.134	*0.017*	**0.000**	**0.001**	**0.000**
z5	0.292	0.292	0.292	0.901	0.945	0.847	0.677	0.677	0.677

wt – wild type; het – heterozygote; mut – mutant; x15, y1-5, z1-5 – coordinates of 5 cephalometric points.
***p*-values < 0.01 are bolded.**

We found the statistically highly significant difference between wild-type and mutant in y2-4, z2 and z4 preoperatively, in z2 and z4 7 days postoperatively, and in y3, z2 and z4 90 days postoperatively, as well as in y2 and y4 between wild-type vs. heterozygote and mutant. The statistically significant difference was found between wild-type and mutant 7 days postoperatively in y3, as well as between wild type vs. heterozygote and mutant preoperatively and 90 days postoperatively, in y3, z2 and z4.

The value of Pd means between the coordinates of each patient and average coordinates, in all of three genotypes of TMTC2 gene [wild type (T/T), heterozygote (T/A), and mutant (A/A)], preoperatively, and 7 and 90 days after the surgery, are presented in Figure 2. We found the highest value of Pd 7 days postoperatively in all of three genotypes, while 90 days postoperatively the value of Pd were lower than preoperatively. Preoperatively, the median was lower than mean in all of the genotypes, while the median was higher than mean 7 and 90 days postoperatively.

The statistical significance of Pd differences between TMTC2 genotypes in all of three scanning times was analyzed using ANOVA and *Post Hoc* Scheffe test. There were no statistically significant differences among the genotypes of TMTC2 gene in all three scanning times.

Using MANOVA, we also tested the statistically significant differences among the coordinates of TMTC2 genotypes in all three scanning times (0, 7, 90 days). There were no statistically significant differences among the genotypes of TMTC2 gene in all three scanning times, for all the coordinates.

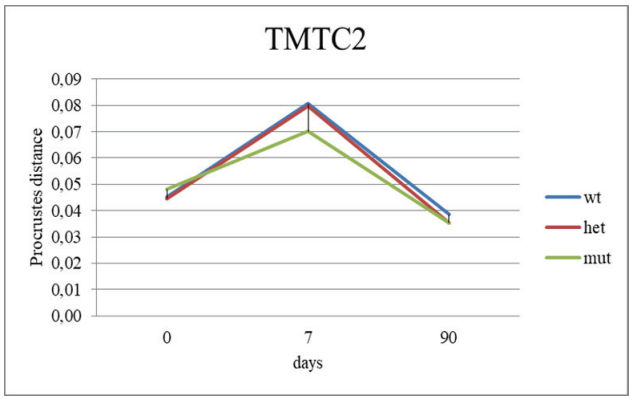

Fig. 2 – Procrustes distances in patients with different TMTC2 genotypes [preoperatively (0), 7 and 90 days postoperatively].
wt – wild type; het – heterozygote; mut – mutant.

Discussion

Previous studies have shown that polymorphisms of PAX3 and TMTC2 genes have a role in determination of the face morphology [11]. The genetic-phenotypic relationship of PAX3 gene is characterized by gene expression in the craniofacial syndrome, as well as in the alveolar rhabdomyosarcoma 2, and in Waardenburg syndrome (types 1 and 3) [12].

In previous studies [1, 4, 12], it was found that PAX3 gene has a role in the endocanthal region growth. This finding provokes the difference in landmark coordinates in this region. As the shape differences are dependent on x, y and z

coordinates of landmarks, it is expected that different genotypes could be associated with differences in the shape of that region [1, 13–15]. On the other hand, there were no studies about associations between PAX3 SNP and x, y and z coordinates of cephalometric points postoperatively.

In our study, we found that there was the statistically highly significant difference in the shape of the examined region between patients with PAX3 wild-type and mutants, preoperatively, while there was no statistically significant difference in the shape between wild-type and heterozygotes as well as between wild-type vs. heterozygotes and mutants. This result showed that PAX3 alleles were expressed differently in facial morphology of the examined region, which was correlated with the expression of the PAX3 gene in Waardenburg syndrome [16–18].

PAX3 gene product is a DNA-binding protein that is expressed during early neurogenesis [19]. Transfection experiments have shown that PAX3 and SOX10 have a direct binding effect for the proximal region of the MITF promoter, which contains sites for both factors [20, 21]. The mutated SOX10 or PAX3 proteins cannot transact this promoter, directly indicating that the two genes directly affect the regulation of MITF expression [22, 23]. Hybridization experiments in dominant mouse megacolon have confirmed that SOX10 dysfunction reduces MITF expression, as well as the development and survival of melanocytes. Authors have suggested that the interaction among three genes that have been altered in Waardenburg syndrome can explain the auditory and pigment symptoms of the disease [24, 25]. MITF and PAX3 gene mutations, encoding transcription factors, are responsible for Waardenburg syndrome 2A [26].

Also, in previous studies, it was found that PAX3 and TMTC2 SNP were associated with cephalometric points' distances in the endocanthal region [1].

Analyzing x, y, and z coordinates of the cephalometric points, we found that there was statistically significant difference between wild-type and mutants, preoperatively in y2-4, z2 and z4, on the basis of which could be concluded that the PAX3 gene has a role in defining the morphology of the medial canthal region, and in the nasion-endocanthion angle. Postoperatively, a statistically significant difference in y2 and y4 was not found. Accordingly, it could be concluded that other factors affected the postoperative change of z2 and z4 and not PAX3 gene polymorphism. This finding could be explained by the influence of surgical intervention on the change in the morphology of the medial canthus due to the concavity of the medial cantus.

The significant differences among shapes of the examined region in relation to genotypes of PAX3 gene were not found postoperatively. As there was no significant difference between wild-type and mutants, postoperatively, we could conclude that there was a role of PAX3 gene to the facial morphometric characteristics, but only preoperatively. As there was no significant correlation between preoperative and postoperative results in general, we could assume that there was no association between PAX3 gene polymorphism and postoperative facial morphometric characteristics.

Also, we did not find any difference in the shape of the examined region among TMTC2 genotypes preoperatively, neither postoperatively [27, 28]. Besides, like in PAX3 gene, there was no correlation between preoperative and postoperative results, so we could suppose that there was no association between TMTC2 gene and facial morphology in the medial cheek region [29].

Postoperative results are based on preoperative morphology, but also are dependent on the postsurgical healing process. Accordingly, we can assume that other factors could affect the changes of three-dimensional coordinates of tested cephalometric points.

Conclusion

Polymorphisms of PAX3 and TMTC2 genes are not associated with changes in the face morphology after the skin tumor excision and direct suture closure of the defect. Other factors might have a role in postoperative changes of three-dimensional coordinates of cephalometric points.

REFERENCES

1. *Patemoster L, Zhurov AI, Toma AM, Kemp JP, St Pourcain B, Timpson NJ*, et al. Genome-wide association study of three-dimensional facial morphology identifies a variant in PAX3 associated with nasion position. Am J Hum Gen 2012; 90(3): 478–85.
2. *Mabuchi F, Mabuchi N, Takamoto M, Sakurada Y, Yoneyama S, Kashiwagi K,* et al. Japan Glaucoma Society Omics Group (JGSOG). Genetic Variant Near PLXDC2 Influences the Risk of Primary Open-angle Glaucoma by Increasing Intraocular Pressure in the Japanese Population. J Glaucoma 2017; 26(11): 963–6.
3. *Madsen MB, Kogelman LJA, Kadarmideen HN, Rasmussen HB.* Systems genetics analysis of pharmacogenomics variation during antidepressant treatment. Pharmacogenomics J 2018; 18(1): 144–52.
4. *Runge CL, Indap A, Zhou Y, Kent JW Jr, King E, Erbe CB,* et al. Association of TMTC2 With Human Nonsyndromic Sensorineural Hearing Loss. JAMA Otolaryngol Head Neck Surg 2016; 142(9): 866–72.
5. *Marenholz I, Esparza-Gordillo J, Rüschendorf F, Bauerfeind A, Strachan DP, Spycher BD,* et al. Meta-analysis identifies seven susceptibility loci involved in the atopic march. Nat Commun 2015; 6: 8804.
6. *Springelkamp H, Mishra A, Hysi PG, Gharahkhani P, Höhn R, Khor CC,* et al. Meta-analysis of Genome-Wide Association Studies Identifies Novel Loci Associated With Optic isc Morphology. Genet Epidemiol 2015; 39(3): 207–16.
7. *Meaike JD, Dickey RM, Killion E, Bartlett EL, Brown RH.* Facial Skin Cancer Reconstruction. Semin Plast Surg 2016; 30(3): 108–21.
8. *Kang SH, Kim MK, An SI, Lee JY.* The effect of orthognathic surgery on the lip lines while smiling in skeletal class III patients with facial asymmetry. Maxillofac Plast Reconstr Surg 2016; 38(1): 18.

9. *Winkelbach S, Molkenstruck S, Wahl FM*. Low-Cost Laser Range Scanner and Fast Surface Registration Approach. In: *Franke K, Müller KR, Nickolay B, Schäfer R*, editors. Pattern Recognition. Conference Proceedings; 28th DAGM Symposium; Berlin, Germany; 2006 September 12–14. Berlin, Heidelberg: Sringer Verlag; 2006, p. 718–28.
10. *Klingenberg CP*. MorphoJ: an integrated software package for geometric morphometrics. Mol Ecol Resour 2011; 11(2): 353–7
11. *Zalc A, Rattenbach R, Auradé F, Cadot B, Relaix F*. Pax3 and Pax7 play essential safeguard functions against environmental stress-induced birth defects. Dev Cell 2015; 33(1): 56–66.
12. *Goulding MD, Chalepakis G, Deutsch U, Erselius JR, Gruss P*. Pax-3, a novel murine DNA binding protein expressed during early neurogenesis. EMBO J 1991; 10(5): 1135–47.
13. *Macina RA, Barr FG, Galili N, Riethman HC*. Genomic organization of the human PAX3 gene: DNA sequence analysis of the region disrupted in alveolar rhabdomyosarcoma. Genomics 1995; 26(1): 1–8.
14. *Lee AS, Yoon N, Gould M, Zhang M*. Dynamic patterns of mononucleated myogenic cell populations in the developing rat hindlimb. Int J Dev Biol 2018; 62(4–5): 303–10.
15. *Lee DH, Ahn SS, Kim JB, Lim Y, Lee YH, Shin SY*. Downregulation of α-Melanocyte-Stimulating Hormone-Induced Activation of the Pax3-MITF-Tyrosinase Axis by Sorghum Ethanolic Extract in B16F10 Melanoma Cells. Int J Mol Sci 2018; 19(6): pii: E1640.
16. *Suzuki N, Mutai H, Miya F, Tsunoda T, Terashima H, Morimoto N*, et al. A case report of reversible generalized seizures in a patient with Waardenburg syndrome associated with a novel nonsense mutation in the penultimate exon of SOX10. BMC Pediatr. 2018 May 23;18(1):171.
17. *Singh AJ, Chang CN, Ma HY, Ramsey SA, Filtz TM, Kioussi C*. FACS-Seq analysis of Pax3-derived cells identifies non-myogenic lineages in the embryonic forelimb. Sci Rep 2018; 8(1): 7670.
18. *Russo I, Di Paolo V, Gurnari C, Mastronuzzi A, Del Bufalo F, Di Paolo PL*, et all. Congenital Rhabdomyosarcoma: a different clinical presentation in two cases. BMC Pediatr 2018; 18(1): 166.
19. *Hanna JA, Garcia MR, Lardennois A, Leavey PJ, Maglic D, Fagnan A,* et al. PAX3-FOXO1 drives miR-486-5p and represses miR-221 contributing to pathogenesis of alveolar rhabdomyosarcoma. Oncogene 2018; 37(15): 1991–2007.
20. *Burri M, Tromvoukis Y, Bopp D, Frigerio G, Noll M*. Conservation of the paired domain in metazoans and its structure in three isolated human genes. EMBO J 1989; 8(4): 1183–90.
21. *Tachibana M, Takeda K, Nobukuni Y, Urabe K, Long J E, Meyers KA,* et al. Ectopic expression of MITF, a gene for Waardenburg syndrome type 2, converts fibroblasts to cells with melanocytes characteristics. Nat Genet 1996; 14(1): 50–4.
22. *Sunryd JC, Cheon B, Graham JB, Giorda KM, Fissore RA, Hebert DN*. TMTC1 and TMTC2 are novel endoplasmic reticulum tetratricopeptide repeat-containing adapter proteins involved in calcium homeostasis. J Biol Chem 2014; 289(23): 16085–99.
23. *Bondurand N, Pingault V, Goerich DE, Lemort N, Sock E, Le Caignec C, et* al. Interaction among SOX10, PAX3 and MITF, three genes altered in Waardenburg syndrome. Hum Molec Genet 2000; 9(13): 1907–17.
24. *Tassabehji M, Read AP, Newton VE, Harris R, Balling R, Gruss P,* et al. Waardenburg's syndrome patients have mutations in the human homologue of the Pax-3 paired box gene. Nature 1992; 355(6361): 635–6.
25. *Tsukamoto K, Nakamura Y, Niikawa N*. Isolation of two isoforms of the PAX3 gene transcripts and their tissue-specific alternative expression in human adult tissues. Hum Genet 1994; 93(3): 270–4.
26. *Barber MC, Cloutier TE, Friedman TB*. PAX3 gene structure, alternative splicing and evolution. Gene 1999; 237(2): 311–9.
27. *Kim K, Heo DW, Kim S, Kim JS, Kim CS, Kang C*. Expansive marker analysis replicating the association of glaucoma susceptibility with human chromosome loci 1q43 and 10p12.31. Eur J Hum Genet 2014; 22(3): 409–13.
28. *Chen LJ, Tam PO, Leung DY, Fan AH, Zhang M, Tham CC*, et al. SNP rs1533428 at 2p16.3 as a marker for late-onset primary open-angle glaucoma. Mol Vis 2012; 18: 1629–39.
29. *Cao D, Jiao X, Liu X, Hennis A, Leske MC, Nemesure B,* et al. CDKN2B polymorphism is associated with primary open-angle glaucoma (POAG) in the Afro-Caribbean population of Barbados, West Indies. PLoS One 2012; 7(6): e39278.

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