Genetic Variability in Slovenian Cohort of Patients with Oculocutaneous Albinism

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Abstract

Oculocutaneous albinism (OCA) is an inherited disorder affecting the visual system and skin pigmentation. Our aim was to evaluate genetic and clinical heterogeneity in a cohort of Slovenian paediatric patients with clinically suspected OCA using advanced molecular-genetics approach. In as much as 20 out of 25 patients, genetic variants explaining their clinical phenotype were identified. The great majority of patients (15/25) had genetic variants in *TYR* gene associated with OCA type 1, followed by variants in *TYRP1*, *SLC45A2* and *HPS1* genes causative for OCA3, OCA4 and Hermansky-Pudlak syndrome type 1, respectively. We concluded that OCA phenotype could not predict genotype and vice versa. Nevertheless, the diagnostic yield after targeted next generation sequencing (NGS) was 80% and proved to be affective in our paediatric cohort of patients with various degree of OCA. Even in 16 patients with normal complexion the diagnostic yield was 62,5%. Interestingly, we have identified a patient of white European ancestry with OCA3, which is an extremely rare report, and one patient with OCA due to the Hermansky-Pudlak syndrome type 1.

Keywords: Oculocutaneous albinism, Hermansky-Pudlak syndrome type 1, next generation sequencing, genetic variant

1. Introduction

Albinism is a rare inherited disorder affecting visual system and skin pigmentation, and has a global incidence of approximately 1 in 17.000.[1,2] The most handicapping manifestations are ocular abnormalities, namely reduced visual acuity with nystagmus, strabismus, photophobia, foveal hypoplasia and misrouting of optic nerve fibres at the chiasm.[3,4] Albinism is clinically classified in three groups. In ocular albinism (OA, OMIM # 300500) pigmentation is impaired only in the eyes, while in oculocutaneous albinism (OCA) also pigmentation of the skin and/or hair is impaired. In syndromic forms, such as syndrome Hermansky-Pudlak and Chediak-Higashi syndrome, additional manifestations are present.[5]

OCA is associated with defective biosynthesis or transport of melanin.[6] Each of the several OCA types is related to the individual genetic defect. OCA1 is caused by disease causing variants in *TYR* gene (OMIM* 606933)[7] encoding enzyme tyrosinase catalysing the first two steps in melanin biosynthesis.[8] In OCA1A subtype, enzyme activity is completely abolished and patients have no pigment and severe ocular symptoms. In OCA1B subtype, residual tyrosinase activity is present and consequently patients may develop some pigment after infancy.[9] Among patients with mild OCA1B, two relatively common *TYR* variants, namely NM_000372.4:c.575C>A (p.Ser192Tyr) and NM_000372.4: c.1205G>A (p.Arg402Gln) located in cis and were reported as a prevalent cause when inherited in trans with pathogenic *TYR* variant.[10–14]

OCA2 is the commonest type and represents about 30% of OCA worldwide.[15] It is caused by disease causing variants in *OCA2* gene (OMIM* 611409) encoding inte-

gral melanosomal transmembrane protein.[16] Patients have various amounts of cutaneous pigment. OCA3 is more common in Africa but is extremely rare in white European or Asiatic populations.[17] It is caused by disease causing variants in *TYRP1* gene (OMIM* 115501) encoding tyrosinase-related protein 1 involved in melanin biosynthesis pathway. OCA4 is caused by disease causing variants in *SLC45A2* gene (OMIM* 606202)[18] encoding melanosomal MATP protein, that might affects tyrosinase activity through the regulation of the melanosomal pH.[19] In 2013, three additional rare OCA types, namely OCA5, OCA6 and OCA7, and their associated genes, were added to the consensus list of albinism types.[2] Recently, additional gene was reported in relation to the oculocutaneous albinism, namely dopachrome tautomerase gene (*DCT*) shown to be associated with autosomal recessive form.[20]

Clinical evaluation of a child with suspected albinism should consist of full clinical examination with special emphasis on nystagmus evaluation, iris transillumination defects detection, foveal hypoplasia gradation and retinal pigment epithelium evaluation.[21] The diagnostic tools of great importance are optical coherence tomography (OCT) for foveal hypoplasia gradation[22] and visual evoked potentials (VEP) which can demonstrate misrouting of optic nerve fibers in the chiasm.[23] Despite significant technological advances in genetic testing, a substantial fraction of individuals with OCA remains genetically unexplained. Missing heritability after common four gene testing was reported to be between 10 and 25% in complete OCA and up to 50% in partial OCA[24] and was still as high as 28% in panel based NGS approach.[12] Nevertheless, next generation sequencing (NGS) based genetic testing presents a possibility for an early definitive diagnosis and management of the disease, especially since OCA types are often difficult to differentiate clinically. Our aim was to evaluate genetic and clinical heterogeneity in a cohort of Slovenian paediatric patients with clinically suspected OCA using combined molecular-genetic approach.

2. Methods

2. 1. Patients

Paediatric individuals with clinical signs of OCA were examined at the outpatient clinic of the Eye Hospital at the University Medical Centre Ljubljana. Clinical ophthalmological examination with visual acuity for distance and near, colour vision, perimetry, ocular motility, biomicroscopy, fundus examination andelectrophysiology was performed. Main albinism signs in the studied group of children were: ocular hypopigmentation with iris translucency or fundus hypopigmentation, foveal hypoplasia, misrouting, nystagmus, skin and hair hypopigmentation. Best corrected monocular and binocular visual acuity was tested using Teller Acuity Cards, Cambridge Acuity Crowding Cards, Lea Symbols Cards; verbal optotypes for

distance and near visual acuity were tested with HOTV optotype and Jaeger tables. Colour vision was assessed at first or follow-up examinations with non-verbal and verbal Ishihara plates. Visual evoked potentials (VEPs) to flash and onset stimulation were recorded in all children[25] at presentation and follow-up, and macular OCT (spectral domain optical coherence tomography) in some cooperative children. OCT images, were obtained in mydriasis with OCT Topcon 3D OCT-1000 (Topcon Medical Systems, Tokyo, Japan) and/or Spectralis HRA + OCT (Heidelberg Engineering, Germany). The retinal thickness and total macular volume were determined using the OCT apparatus software.

All participants or parents of minors gave their written informed consent prior to the study (approved by the Republic of Slovenia National Medical Ethics Committee nr. 132/03/15) and the study followed the statement of the Republic of Slovenia National Medical Ethics Committee nr. 0120–489/2018/7 and principles of the Declaration of Helsinki.

2. 2. Genetic Testing

Genetic testing was performed at the Clinical Institute for Special Laboratory Diagnostics of the University Children's Hospital at the University Medical Centre Ljubljana. Genomic DNA was isolated from peripheral blood samples with FlexiGene DNA Kit 250 (Qiagen, Hilden, Germany). In male patients, ocular albinism due to *GPR143* gene variants was previously excluded.[26] To evaluate the genetic aetiology of ocular albinism, we performed targeted NGS with TruSightOne Sequencing Panel on the MiSeq platform desktop sequencer coupled with MiSeq Reagent kit v3 (all Illumina, USA). Following on-board primary analysis, we performed secondary data analysis with Variant Studio 2.3 software (Illumina, USA). Rare variants with minor allele frequency less than 5% in genes reported to be related to syndromic and non-syndromic OCA (*AP3B1, BLOC1S3, BLOC1S6, C10ORF11, GPR143, HPS1, HPS3, HPS4, HPS5, HPS6, LYST, MC1R, MITF, MLPH, MYO5A, OCA2, RAB27A, SLC24A5, SLC45A2, TYR, TYRP1*) were further evaluated. Possibly causative variants were confirmed by targeted Sanger sequencing using custom oligonucleotides, BigDye Terminator v3.1 sequencing kit, and ABI Genetic Analyser 3500 (both Applied Biosystems, USA).

The pathogenicity of the variants was evaluated as recommended by the American College of Medical Genetics (ACMG)[27], while novel variants were evaluated with ensemble *in silico* prediction tools CADD,[28] REVEL,[29] VEST4[30] and SpliceAI,[31] while their frequency in general population was assessed using GnomAd database.[32] Additionally, we evaluated large deletions and duplications of the *OCA2* and *TYR* genes with multiplex ligation-dependent probe amplification (MLPA). The probe mix SALSA MLPA P325 OCA2 (MRC-Holland, The Netherlands)

was used according to the manufacturer's instructions. We included three normal control samples to normalize for the allele dosage. We separated amplification products with capillary electrophoresis on ABI Genetic Analyser 3500 (Applied Biosystems, USA) and analysed raw data with GeneMapper® Software Version 4 (Life Technologies, USA). Peak patterns were evaluated using Coffalyser v8 software (MRC-Holland, The Netherlands).

3. Results

3. 1. Clinical Characteristics

Altogether, 25 paediatric individuals, median age 12 years (age range 5–19), 16 male/9 female from 24 un-

related families with clinical signs of OCA were included in the study; patients 12* and 13* were brothers. Among them, 16 had normal complexion in regard to the family members. Their clinical characteristics were summarised in the Table 1. All patients had horizontal and/or rotary nystagmus. Patients had no clear deficit of near and colour vision, their corrected vision for distance was within limits of mild amblyopia. The patients' best corrected visual acuity for distance was 0.2–0.6 Snellen equivalent (0.7–0.2 log MAR). Perimetry revealed no evident lesions of the visual pathways. Electrophysiological evaluation (VEP) showed contralateral asymmetry in all studied children, but not being apparent in the control (Figure 1). Retinal pigmentation was normal or with rare retinal pigment epithelial pigmentation at the posterior pole or retinal vessels, and

Table 1: Clinical finding in children with suspected OCA. M-male, F-female. *Iris translucency*: – not present; 1/3 one third; 2/3 two thirds; 3/3 total. *Foveal pit* : ± underdeveloped; + minimal foveal depression; ++ absent; +++ no foveal depression, choroidal vessels seen at the posterior pole and foveal area. *Retinal pigmentepithelial pigmentation*: – changes not evident; ± rare, abnormal periphery; rare +, choroidal vessels seen at the posterior pole, but not in the foveal area; rare ++, choroidal vessels seen also in the foveal area. *Refraction:* HA – hypermetropic astigmatism, low (≤ 2.50 D, Dcyl); medium (> 2.5 D, Dcyl); high myopia (–10 and –11 D). *Photophobia:* + minimal outside; ++ in light condition outside, inside; +++ in normal light, * siblings.

Pro-band	Gender	Skin pigmentation in the context of the family	Iris translucency	Foveal pit	Retinal pigment epithelial pigmentation	Refraction	Photophobia	Classification according to the genetic defect
1	M	unremarkable	1/3	+	–	Low HA	–	OCA1
2	F	unremarkable	1/3	±	–	Low HA	–	
3	M	unremarkable	1/2	+	–	Low HA	–	
4	M	unremarkable	1/3	+	Rare +	Medium HA	+	
5	F	unremarkable	1/3	+	Rare +	Low HA	–	
6	F	unremarkable	1/3	+	Rare +	Low HA	–	
7	M	fair	2/3	++	Rare +	Medium HA	++	Highly likely OCA1
8	F	unremarkable	–	+	Rare +	Low HA	–	
9	F	unremarkable	–	–	Rare +	Low HA	–	
10	M	unremarkable	1/3	+	Rare +	Low HA	–	
11	M	fair	1/3	+	–	Medium HA	–	
12*	M	fair	–	+	Rare +	Low HA	–	
13*	M	fair	1/3	+	Rare +	Low HA	–	
14	F	unremarkable	1/3	+	Rare +	Medium HA	–	
15	M	fair	–	++	Rare +	Medium HA	–	
16	F	unremarkable	1/3	+	Rare +	Medium HA	+	OCA3
17	M	fair	–	+	Rare +	Medium HA	–	
18	M	fair	2/3	++	Rare +	Medium HA	–	OCA4
19	M	fair	3/3	+	Rare +	Medium HA	+	
20	F	fair	1/3	+++	Rare ++	Low HA	+	Hermansky-Pudlak syndrome type 1
21	M	unremarkable	1/3	+	Rare ±	Low HA	++	?
22	M	unremarkable	–	–	Rare +	Medium HA	–	
23	M	unremarkable	1/3	+++	Rare +	High myopia	–	
24	F	unremarkable	1/3	±	Rare ±	Low HA.	+	
25	M	unremarkable	1/3	+	–	Low HA	–	
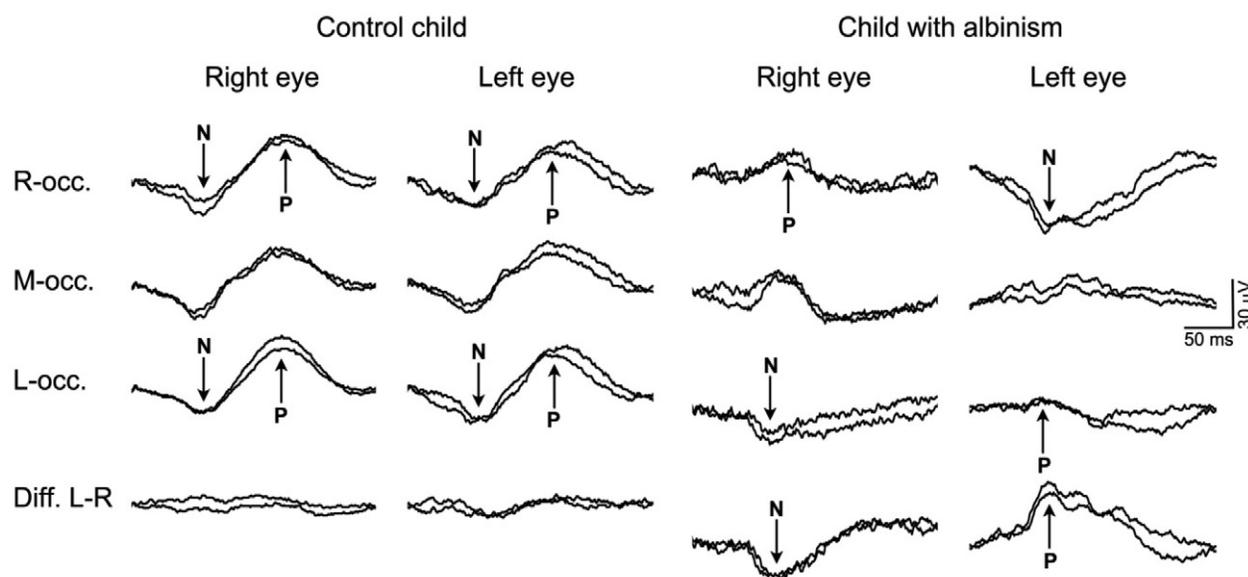
Figure 1: Flash VEP from a control child and child with albinism. In a control child there is a symmetrical distribution of the P and N waves over the lateral two electrodes (R-occ.: right occipital electrode, L-occ.: left occipital electrode) and no deflections of polarity on the differential channel (Dif. L-R), representing the difference in potentials between the left and right electrodes (subtraction of the right from the left occipital signal). In a child with albinism, there is a marked asymmetry over the lateral two electrodes, with the waves of opposite were compared between the eyes (albino crossed asymmetry). From the right eye a positive (P) wave is seen over the right electrode and a negative (N) wave is above the left electrode, while from the left eye the distribution of P and N waves is exactly the opposite. This asymmetry is even more clearly seen on a differential channel, where predominance on the negative wave is seen on the right eye, and a positive one on the left eye.

evident macular hypoplasia, but normal optic discs. Complexion, hair and lashes pigmentation did not differ among individual participants. None of the children had really dark or red complexion or hair, iris pigmentation was blue to green, none of the included children had brown iris.

At the time of the first referral to the genetic testing, no additional signs or symptoms were reported. However, at the time when the genetic results were issued in 2018 and results led to the diagnosis of the Hermansky-Pudlak syndrome type 1, patient 20 she was already referred to the gastroenterological assessment by her paediatrician because of the chronic abdominal pain and occasional diarrhoea. Fulminant Crohn's disease with perianal fistulas was diagnosed and treated with infliximab (Remicade). According to the clinical practice, she was also referred to the paediatric haematology department where prolonged bleeding due to thrombocytopathy was recognised.

3. 2. Genetic Testing

Genetic characteristics of the cohort were summarised in Table 2. Patients 1 to 6 had pathogenic variants on both alleles of the *TYR* gene causative for OCA1. Additionally, probands 7 to 15 had variants on both alleles of the *TYR* gene highly suspected to be causative for at least mild form of OCA1. Patients 16 and 17 had pathogenic variants on both alleles of the *TYRP1* gene causative for OCA3. Patients 18 and 19 had pathogenic variants on both alleles of the *SLC45A2* gene causative for OCA4, while patient 20 had pathogenic variants on both alleles of the *HPS1* gene causative for Hermansky-Pudlak syndrome type 1. Patients 21 to 25 carried monoallelic variants that could not fully explain the clinical presentations. Parental analysis confirmed the segregation of the detected pathological variants.

Among the genetic variants detected in the cohort, three variants, namely *TYR* NM_000372.4: c.1430G>A, p.Trp477Ter; *SLC45A2* NM_016180.4: c.302G>A, p.Arg101His; *TYRP1* NM_000550.2: c.913+1G>A were not previously reported in patients with OCA and were predicted to be deleterious. Their general population data and *in silico* prediction scores are summarised in the Table 3. *SLC45A2* missense variant NM_016180.4: c.302G>A; p.Arg101His was detected in homozygous state in patient 19. Nonsense variant *TYR* NM_000372.4: c.1430G>A; p.Trp477Ter introducing premature termination codon was detected in patients 3 and 10 in compound heterozygous state with another monoallelic variant in *TYR* gene. Intronic *TYRP1* variant located in consensus splice donor site (*TYRP1*, NM_000550.2: c.913+1G>A) was detected in patient 21 in heterozygous state, while variant on the other *TYRP1* allele has not been detected.

Diagnostic yield after the targeted NGS sequencing was 80% (20/25). MLPA analysis did not reveal large deletions or duplications in analysed regions of *OCA2* or *TYR* genes, as detected height ratios of the fluorescent peaks were in the normal height ratio range between 0.7–1.3.

Table 2: Genetic variants detected in OCA patients (novel variants are in bold; M: male; F: female; homo: homozygous variant; hetero: heterozygous variant; PA-paternal allele, MA-maternal allele, ? segregation analysis was inconclusive; * siblings.).

Proband	Variant		ACMG classifi-cation [27]	HGMD Professional 2021.1[31]	ClinVar[33]
1/M	*TYR* NM_000372.4: c.1A>G (NP_000363.1: p.Met1?)	Homo	Likely path.	OCA1, CM981972	Pathogenic
	TYR NM_000372.4: c.1205G>A (NP_000363.1: p.Arg402Gln)	Homo	Benign	OCA1, CM971555	Conflic. int. path
2/F	*TYR* NM_000372.4: c.650G>A (NP_000363.1: p.Arg217Gln)	Homo	Path.	OCA1, CM930714	Likely path.
	TYR NM_000372.4: c.1205G>A (NP_000363.1: p.Arg402Gln)	Homo	Benign	OCA1, CM971555	Conflic. int. path
3/M	*TYR* NM_000372.4: c.650G>A (NP_000363.1p.Arg217Gln)	Het,MA	Path.	OCA1, CM930714	Likely path.
	***TYR* NM_000372.4: c.1430G>A (NP_000363.1p.Trp477Ter)**	Het,PA	Likely path.	Not reported	Not reported
4/M	TYR NM_000372.4: c.265T>C (NP_000363.1: p.Cys89Arg)	Het MA	Path.	OCA1, CM910381	Pathogenic
	TYR NM_000372.4: c.1352A>G (NP_000363.1: p.Tyr451Cys)	Het PA	Likely path.	OCA1, CM117403	Likely path.
	TYR NM_000372.4: c.1217C>T (NP_000363.1: p.Pro406Leu)	Het MA	Likely path.	OCA1,CM910385	Likely path.
5/F	TYR NM_000372.4: c.265T>C; (NP_000363.1: p.Cys89Arg)	Het	Path.	OCA1, CM910381	Pathogenic
	TYR NM_000372.4: c.325G>A (NP_000363.1: p.Gly109Arg)	Het	Likely path.	OCA1, CM13052	Likely path.
	TYR NM_000372.4: c.1352A>G (NP_000363.1: p.Tyr451Cys)	Het	Likely path.	OCA, CM117403	Likely path.
6 /F	*TYR* NM_000372.4: c.1A>G (NP_000363.1: p.Met1?)	Het PA	Likely path.	OCA1, CM981972	Pathogenic
	TYR NM_000372.4: c.1217C>T (NP_000363.1: p.Pro406Leu)	Het MA	Likely path.	OCA1, CM910385	Likely path.
	TYR NM_000372.4: c.1205G>A (NP_000363.1: p.Arg402Gln)	Het PA	Benign	OCA1, CM971555	Conflic. int. Path
	TYR NM_000372.4: c.575C>A (NP_000363.1: p.Ser192Tyr)	Het MA	Benign		Benign
7/M	*TYR* NM_000372.4: c.265T>C (NP_000363.1: p.Cys89Arg)	Het MA	Path.	OCA1, CM910381	Path
	TYR NM_000372.4: c.1352A>G (NP_000363.1: p.Tyr451Cys)	Het MA	Likely path.	OCA, CM117403	Likely path.
	TYR NM_000372.4: c.1205G>A (NP_000363.1: p.Arg402Gln)	Het PA	Benign	OCA1, CM971555	Conflic. int. path.
	TYR NM_000372.4: c.575C>A (NP_000363.1: p.Ser192Tyr)	Het (?)	Benign		Benign
8/F	*TYR* NM_000372.4: c.1063G>C (NP_000363.1: p. Ala355Pro)	Het PA	Path.	OCA1, CM971550	Pathogenic
	TYR NM_000372.4: c.1217C>T (NP_000363.1: p.Pro406Leu)	Het PA	Likely path	OCA1, CM910385	Likely path.
	TYR NM_000372.4: c.1205G>A (NP_000363.1: p.Arg402Gln)	Het MA	Benign	OCA1, CM971555	Conflic. int. path.
	TYR NM_000372.4: c.575C>A (NP_000363.1: p.Ser192Tyr)	Het MA	Benign		Benign
9/F	TYR NM_000372.4: c.265T>C; (NP_000363.1: p.Cys89Arg)	Het PA	Path.	OCA1, CM910381	Pathogenic
	TYR NM_000372.4: c.1352A>G (NP_000363.1: p.Tyr451Cys)	Het PA	Likely path.	OCA, CM117403	Likely path.
	TYR NM_000372.4: c.1205G>A (NP_000363.1: p.Arg402Gln)	Het MA	Benign	OCA1, CM971555	Conflic. int. path
10 /M	***TYR* NM_000372.4: c.1430G>A (NP_000363.1p.Trp477Ter)**	Het PA	Likely path.	Not reported	Not reported
	TYR NM_000372.4: c.1205G>A (NP_000363.1: p.Arg402Gln)	Het MA	Benign.	OCA1, CM971555	Conflic. int. Path
	TYR NM_000372.4: c.575C>A (NP_000363.1: p.Ser192Tyr)	Het (?)	Benign		Benign
11/M	*TYR* NM_000372.4: c.650G>A (NP_000363.1: p.Arg217Gln)	Het	Path.	CM930714	Likely path.
	TYR NM_000372.4: c.1205G>A (NP_000363.1: p.Arg402Gln)	Homo	Benign	CM041478	Conflic. int. path
12*/M	*TYR* NM_000372.4: c.650G>A (NP_000363.1: p.Arg217Gln)	Het	Path.	OCA1, CM930714	Likely path.
	TYR NM_000372.4: c.1205G>A (NP_000363.1: p.Arg402Gln)	Homo	Benign	OCA1, CM971555	Conflic. int. Path
	TYR NM_000372.4: c.575C>A (NP_000363.1: p.Ser192Tyr)	Het	Benign		Benign
13*/M	*TYR* NM_000372.4: c.650G>A (NP_000363.1: p.Arg217Gln)	Het	Path.	OCA1, CM930714	Likely path.
	TYR NM_000372.4: c.1205G>A (NP_000363.1: p.Arg402Gln	Homo	Benign	OCA1, CM971555	Conflic. int. Path
	TYR NM_000372.4: c.575C>A (NP_000363.1: p.Ser192Tyr)	Het	Benign		Benign

Proband	Variant		ACMG classification [27]	HGMD Professional 2021.1 [31]	ClinVar [33]
14/F	TYR NM_000372.4: c1217C>T (NP_000363.1: p.Pro406Leu)	Het PA	Likely path	OCA1, CM910385	Likely path.
	TYR NM_000372.4: c.1205G>A (NP_000363.1: p.Arg402Gln)	Het MA	Benign	OCA1, CM971555	Conflic. int. Path
	TYR NM_000372.4: c.575C>A (NP_000363.1: p.Ser192Tyr)	Homo	Benign		Benign
15/M	TYR NM_000372.4: c.1217C>T (NP_000363.1: p.Pro406Leu)	Het	Likely path	OCA1, CM910385	Likely path.
	TYR NM_000372.4: c.1205G>A (NP_000363.1: p.Arg402Gln)	Het	Benign	OCA1, CM971555	Conflic. int. Path
	TYR NM_000372.4: c.575C>A (NP_000363.1: p.Ser192Tyr)	Homo	Benign		Benign
16/F	*TYRP1*, NM_000550.2: c.670C>T (NP_000541.1:p.His224Tyr)	Homo	Likely path.	Not reported	Likely path.
17 /M	*TYRP1* NM_000550.2: c.70G>A (NP_000541.1: p.Ala24Thr	Het	Benign	OCA3: CM135782	Conflic. int. path
	TYRP1 NM_000550.2: c.418G>T (NP_000541.1: p.Glu140Ter)	Het	Pathogenic	OCA3: CM172531	Not reported
18/M	*SLC45A2* NM_016180.4: c.606G>C (NP_057264.3:p.Trp202Cys)	Homo	Likely path.	OCA4: CM040231	Conflic. int. Path/ Likely path.
19/M	*SLC45A2* **NM_016180.4: c.302G>A (NP_057264.3:p. Arg101His)**	Homo	Likely path.	Not reported	Not reported
	TYR NM_000372.4: c. 589G>A (NP_000363.1:p.Asp197Asn)	Het	VUS	Not reported	Not reported
20/F	*OCA2* NM_000275.2: c.1025A>G (p.Tyr342Cys)	Het	Likely path.	OCA, CM091279	Likely path./ VUS
	HPS1, NM_000195.3: c.972dupC (NP_000186.2:p.Met325HisfsTer128)	Het	Pathogenic	Hermansky-Pudlak, CI962292	Likely path
	HPS1, NM_000195.3: c 1189delC (NP_000186.2:p.Gln397SerfsTer2)	Het	Pathogenic	Hermansky-Pudlak, CD982692	Likely path
21/M	***TYRP1*, NM_000550.2: c.913+1G>A**	Het	Likely path.	Not reported	Not reported
22/M	TYR NM_000372.4: c.1205G>A (NP_000363.1p.Arg402Gln)	Het	Benign	OCA1, CM971555	Conflic. int. path
	TYR NM_000372.4: c.575C>A (NP_000363.1: p.Ser192Tyr)	Homo	Benign		Benign
23/M	TYR NM_000372.4: c.1205G>A (NP_000363.1p.Arg402Gln)	Het	Benign	OCA1, CM971555	Conflic. int. path
24/F	*TYR* NM_000372.4: c.575C>A (NP_000363.1: p.Ser192Tyr)	Het	Benign		Benign
25/M	*TYR* NM_000372.4: c.575C>A (NP_000363.1: p.Ser192Tyr)	Homo	Benign		Benign

Table 3: General population frequencies and *in silico* prediction scores of the novel genetic variants detected in OCA patients (REVEL[29], VEST4[30] and SpliceAI[31] values O-1, and CADD[28] Phred values: variants with higher scores are predicted to be more likely pathogenic; nd: not detected)

Variant	Position and MAF (gnomAD[32])	*In silico* prediction tools and scores
TYR NM_000372.4: c.1430G>A (NP_000363.1p.Trp477Ter)	nd	CADD: 39 VEST4:0,88
SLC45A2 NM_016180.4: c.302G>A (NP_057264.3:p. Arg101His)	chr5:33984282: rs763531791 MAF: A = 0,0024% (6/251,262 alleles)	CADD: 36,6 REVEL:0.829 VEST4: 0.65
TYRP1, NM_000550.2: c.913+1G>A	chr9:12698656: rs748926400 MAF: A = 0,0016% (4/250,176 alleles)	CADD: 34 SpliceAI: 0.85

4. Discussion

OCA shows considerable clinical heterogeneity.[2] Consequently, individual types of albinism might be difficult to differentiate clinically in children, especially in those with light complexion, where there might be an overlap with other related disorders. Even though OCA is a genetically heterogeneous disorder, NGS based genetic testing enables timely definitive etiological diagnosis and consequently appropriate management of the disease. This is of notable importance in cases with mild or partial clinical manifestations.[10]

Among 25 paediatric patients of Slovenian descent with clinically suspected OCA, as much as 16 had normal complexion in regard to the other family members, and some of them had only mild albinism-related features (Table 1). In as much as 20 out of 25 patients (80%) in the entire cohort, genetic variants explaining their clinical phenotype were identified (Table 2). This is in accordance with other recent reports, where molecular diagnosis was achieved in 92% of patients with mild partial albinism[10] and 72,3% of patients with albinism.[12] Great majority of patients in our cohort (60% – 15/25) had *TYR* disease causing variants. This is surprisingly high when compared to the large cohort of 990 tested patients in France, where the number was 41,8%[12]. High frequency of *TYR* disease causing variants can partly be explained with the fact that ocular albinism due to *GPR143* variants was previously excluded from our group of patients.[26] For instance in above mentioned large cohort *GPR143* variants were responsible for 7% of the cases.[12] Among our remaining patients, 4% (1/25) had only monoallelic disease causing variants that cannot fully explain the clinical presentation, while in 16% (4/25) no disease causing variant was detected in analysed genes. This is comparable with the large reported cohort, where 12% of patients had monoallelic variants and 15,5% had no detected disease causing variants.[12] Surprisingly, we did not identify variants in *OCA2* gene that would be a probable cause of albinism in our cohort. We identify only one *OCA2* variant in heterozygous state in a patient 20 carrying *HPS1* disease causing variants. NGS results in this study were confirming the results of our earlier testing approach using selective gene Sanger sequencing. This is in concordance with a previously reported group of patients with partial OCA, where OCA2 disease causing variants were identified only in heterozygous state together with the heterozygous variant in *TYR* gene.[10]

The novel *TYR* NM_000372.4: c.1430G>A (NP_000363.1 p.Trp477Ter) variant was detected in patients 3 and 10 in compound heterozygous state with another *TYR* variant. This variant is introducing premature termination codon and was predicted to be pathogenic. Both patients harbouring this variant had mild signs of OCA with normal complexion, mild to medium iris transillumination, foveal depression still present, but smaller, normal or rare retinal pigment epithelium, and no photo-

phobia. The mild phenotypic characteristics in patient 10 are likely associated with hypomorfic variant present on the other *TYR* gene allele as it was previously reported.[14]

SLC45A2 variant NM_016180.4: c.302G>A (NP_057264.3: p.Arg101His) was detected in homozygous state in patient 19. It has so far not been reported in OCA and was predicted to be pathogenic. A different amino acid change, namely p.Arg101Cys, on the same position as here reported variant, was previously reported in a patient with OCA4, but his clinical characteristics were not described in more details (HGMD acc. nr. CM083847). Variants in *SLC45A2* gene are associated with OCA4. This is a rare OCA type, reported in approximately 3% of European patients,[34–36] but is more common in Japan.[37] OCA4 is clinically variable and overlapping with other OCA types.[5] Patient 19 had mild clinical phenotype with fair complexion, minimal foveal depression, rare retinal pigment epithelium, choroidal vessels seen at the posterior pole, but not in the foveal area, medium hypermetropic astigmatism, and minimal photophobia. His clinical presentation did not differ significantly from other patients, nevertheless, he was the only patients in this group with complete iris transillumination.

Variants in *TYRP1* gene are associated with OCA3, an extremely rare type in Caucasian patients, reported only in individual patients[17] and in 2,1% of patients in a cohort of mainly but not exclusively French origin.[12] Patient 16 had homozygous *TYRP1* variant NM_000550.2:c.670C>T (NP_000541.1:p.His224Tyr) that was previously reported in ClinVar database in a patient with ocular albinism (Allele ID: 360904) but was so far not reported in Human Gene Mutation Database. He had normal complexion, mild iris transillumination, choroidal vessels seen at the posterior pole, but not in the foveal area, medium hypermetropic astigmatism, and minimal photophobia. His clinical presentation did not differ significantly from other patients and was not concordant with the reported Caucasian patients, who had light-yellow skin, yellow-gold hair with orange highlights and fair eyelashes, divergent strabismus, and no photophobia.[17]

Among the variants that were classified as highly susceptive for OCA was also the *TYR* gene harbouring two allelic variants, namely NM_000372.4:c.575C>A (p.Ser-192Tyr) and NM_000372.4: c.1205G>A (p.Arg402Gln). Those variants individually are frequently reported in general population and consequently regarded as benign. Nevertheless, when these are located *in cis* they are much rarer and when inherited *in trans* with pathogenic *TYR* variant, they were repeatedly reported to be causative for mild or partial form of OCA.[10–13] This complex *TYR* allele was present in several patients in our cohort, while in six patients (subjects 8, 10, 12, 13, 14, 15) it was inherited in *trans* with other *TYR* variant. Therefore, those patients we classified as likely OCA1. They all had normal or fair complexion, rare retinal pigment epithelium, low or medium hypermetropic astigmatism, and no photophobia – con-

cordant with mild OCA. Among them, two were brothers (subject 12 and 13) with very similar clinical presentation. They only differed in iris transillumination that was present in mild form only in the younger brother (subject 13). Similarly as in our cohort, this complex allele was the most common disease causing allele in a previously reported group of patients with partial OCA.[10]

Patient 20 was compound heterozygote for two known disease causing variants in *HPS1* gene associated with Hermansky-Pudlak syndrome type 1. Additionally, she was a heterozygous carrier of know disease causing variant in *OCA2* gene associated with OCA2. In our cohort, she had the most severe retinal pigment epithelium phenotype with choroidal vessels seen also in the foveal area. She had fair complexion, mild iris transillumination, no foveal depression, low hypermetropic astigmatism, and photophobia. She was referred to genetic testing due to her albinism-related symptoms and the genetic testing results led to the diagnosis of the Hermansky-Pudlak syndrome type 1 in 2018 when she was 11 years old. Later on, fulminant Crohn's disease with perianal fistulas and prolonged bleeding due to thrombocytopathy were recognised, both known manifestations of Hermansky-Pudlak syndrome.[38,39] The diagnostic pathway that led to the diagnosis of Hermansky-Pudlak syndrome in this patient confirms the importance of genetic testing in children with albinism. As previously suggested, early identification of the genetic aetiology of albinism can reveal serious syndromic causes of albinism that need appropriate clinical management including clinical evaluation of possible extraocular manifestations.[40]

In our cohort of patients, phenotype could not predict genotype or vice versa, and there were no specific clinical signs correlating with the molecular diagnosis. Nevertheless, these children seem to be of lighter complexion with greater foveal hypoplasia and rare retinal pigment epithelium. Iris transillumination did not correlate with photophobia or other clinical signs, but it was present to a certain extent in all subjects. Furthermore, all subjects had low to medium hypermetropic astigmatism, with one who was highly myopic.

In conclusion, NGS based genetic testing had a high diagnostic yield of 80% in our paediatric cohort of patients with various degrees of OCA and previously excluded OA due to disease causing variants in *GPR143* gene. Interestingly, we have identified a patient of white European ancestry with OCA3, which is an extremely rare report, and one patient with OCA due to the Hermansky-Pudlak syndrome type 1.

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Declaration of interest:

There is no conflict of interest declared.

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Povzetek

Okulokutani albinizem (OCA) je dedna motnja, ki vpliva na vidni sistem in pigmentacijo kože. Naš cilj je bil z uporabo naprednega molekularno-genetskega pristopa oceniti genetsko in klinično heterogenost v kohorti slovenskih pediatričnih pacientov s klinično domnevanim OCA. Pri 20 od 25 pacientov so bile ugotovljene genetske različice, ki pojasnjujejo njihov klinični fenotip. Velika večina pacientov (15/25) je imela genetske različice gena *TYR*, povezanega z OCA tipa 1, sledile so mu različice genov *TYRP1, SLC45A2* in *HPS1*, ki so vzrok za OCA3, OCA4 in Hermansky-Pudlakov sindrom tipa 1. Ugotovili smo, da fenotip OCA ne more napovedati genotipa in obratno. Kljub temu je bil diagnostični izkoristek po ciljanem sekvenciranju naslednje generacije (NGS) 80 % in se je izkazal za učinkovitega v naši pediatrični kohorti pacientov z različno stopnjo OCA. Tudi pri 16 pacientih z normalno poltjo je bil diagnostični izkoristek 62,5 %. Zanimivo je, da smo identificirali pacienta belega evropskega porekla z OCA3, kar je izjemno redko poročano, in enega pacienta z OCA zaradi Hermansky-Pudlakovega sindroma tipa 1.