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Phenotypic and Genetic Features that Differ Between Hereditary and Sporadic Melanoma: Results of a Preliminary Study from a Single Center from Turkey

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ABSTRACT

Introduction: Most melanoma patients under our supervision lack characteristic phenotypic features for melanoma. In contrast, history of cancers other than melanoma and early age at onset were common. This observation was in favor of hereditary melanoma.

Objectives: To search for the phenotypic and genetic features that differ between sporadic and hereditary melanomas.

Methods: In order to reveal phenotypic features, detailed physical exam was conducted to all melanoma patients (N = 43) and for genetic features. CDKN2A and MC1R mutations were detected with Sanger sequencing method. Assignment to hereditary and sporadic groups was done according to the "melanoma cancer syndrome assessment tool". Patients who were diagnosed before the age of 50 were also assigned to the hereditary melanoma group.

Results: Thirty-one patients were assigned to the hereditary group and 12 to the sporadic group. Fair eye color was statistically significantly higher in the sporadic group (P = 0.000). CDKN2A was detected in only 1 patient in the hereditary group. MC1R mutations were found in 12 out of 13 (92.3%) in the hereditary group with a score ≥3 points, 13 out of 18 (72.2%) in the early age at onset group and 5 out of 12 (41.7%) in the sporadic group (P = 0.024).

Conclusions: Incidence of CDKN2A mutations in our hereditary group is in accordance with the reported incidences from Mediterranean countries. The difference between the hereditary and sporadic groups in terms of MC1R mutations supports the idea that MC1R genetic testing might help to determine patients with higher risk for hereditary melanoma.

Introduction

Cutaneous melanoma is a malignant solid tumor that arises from melanocytes in the skin. Melanoma accounts for approximately 2-5% of all skin cancers, but is the most common cause of skin cancer-related deaths. If melanoma is diagnosed early, it can be treated by simple excision, thus cancer-related morbidity and mortality may be prevented [1]. Therefore, it is important to identify groups at high risk for melanoma development. Three main risk factors namely, environmental factors, phenotypic features and genetic features and intermittent intense sun exposure play role in melanoma development. The most common environmental risk factor for the development of melanoma is intermittent intense sun exposure [2]. Fair skin, fair hair and eye color, freckles, multiple solar lentigines, tendency to sunburn and inability to tan are the best-known phenotypic risk factors. Approximately 5-10% of melanomas develop due to genetic factors and this type of melanomas are called hereditary melanomas [3]. Genes associated with melanoma range from rare but high-penetrating tumor suppressor genes such as cyclin-dependent kinase inhibitor 2A (CDKN2A), to very common but medium-penetrating genes such as the melanocortin 1 receptor gene (MC1R). The first gene described as a risk factor for hereditary melanoma is CDKN2A. Germline mutations in the CDKN2A gene are responsible for 40% of the hereditary melanoma patients [4,5]. MC1R normally determines the hair and skin color and can lead to melanoma development through both pigment-related and non-pigment-related pathways [6]. MC1R increases the penetration of CDKN2A from 50% to 84% thus called the "melanoma modifier gene" [7].

Leachman et al suggested that in addition to melanoma-dominant pattern of inheritance like CDKN2A gene mutations, melanoma can also be a part of other cancer syndromes like hereditary breast ovarian cancer syndrome, Li Fraumeni syndrome, xeroderma pigmentosum, phosphatase and tensin homolog (PTEN) hamartoma syndrome. They suggested that patients with a family history of pancreatic cancers, neurologic cancers, renal cell carcinoma and/or mesothelioma should rise suspicion for these cancer syndromes [8]. This overlap might likely to have major implications for genetic testing. It is possible that families that fail to meet the genetic testing criteria for a melanoma-dominant syndrome, still may carry an increased risk for melanoma cancer syndromes. Leachman et al. offered a comprehensive cancer gene assessment tool for families with a hereditary pattern of cancer that includes melanoma [8].

The incidence of melanoma in Turkey according to the Ministry of Health Unified Database in 2014 is 1.8 per 100000 in men and 1.2 per 100000 in women which might be considered as quite low when compared with the incidence of melanoma in Europe where it ranges from 2.2 to 19.2 per 100000 people [9,10]. Interestingly, we have observed that most of the melanoma patients under our supervision had dark hair, darker skin color and had less sun exposure than expected for melanoma development. In contrast, we have realized that most of them had personal and/or family history of cancers other than melanoma and/or were diagnosed before 50 years of age. Thus, we have hypothesized that, in our patient group genetic risk factors rather than phenotypic features may play a more important role at the development of cancer/melanoma.

Objectives

The aim of this study was to define the phenotypic and genetic features that differentiate hereditary melanomas from sporadic melanomas.

Methods

After the approval of the ethics committee, 43 histologically confirmed patients with cutaneous melanoma were recruited for this prospective study between February 2017 and October 2017 at the Department of Dermatology, University of Hacettepe Faculty of Medicine, Ankara, Turkey.

A face-to-face questionnaire was applied to all patients. After the dermatological examinations of the patients were completed, a whole-body nevus examination was performed

using the Dermlite® hand dermatoscope, and then the nevi were recorded using the Fotofinder® Universe Version 2.0.39.3- (X64) digital dermatoscope.

Sociodemographic features (age, sex, occupation, place of birth, place of current residence, phenotypic features (hair color, eye color, skin phototype and presence of freckles), treatments taken before to the diagnosis of melanoma (whether the patients received immunosuppressive therapy and/or any phototherapy such as narrowband ultraviolet B, psoralen ultraviolet A (PUVA) and /or local PUVA therapy), sun exposure (the patients history of working outdoors and its duration, whether they participated in outdoor sports and similar activities, the habit and duration of vacation in sunny holiday resorts, the habit and duration of sunbathing, the history and age of second-degree sunburns, the history and age of indoor tanning, the characteristics of sun protection habits, including using sunscreen), genetic factors (the personal history and family history including the presence of melanoma and other cancers in the individual and/or their first and second degree relatives) were questioned and recorded in the questionnaire form.

The stage of melanoma at diagnosis, anatomical location of melanoma, histopathological features of melanoma (Breslow thickness, presence of ulceration, presence and number of mitoses, presence of lymphocytic infiltration, late regression, presence of lympho-vascular invasion) were noted on a separate sheet, namely the examination form. The presence of re-excision after diagnosis and/or sentinel lymph node biopsy were recorded at the examination form. Total number of nevi, anatomic location of the nevi, number of typical and atypical nevi, number and size of giant congenital melanocytic nevi, number of actinic keratosis, presence of solar lentigo, presence of photo aging, presence and number of pigmented lesions in the iris on inspection were recorded on the examination form. Body mass index was calculated for each patient. Additional findings such as benign skin tumors (eg cherry angiomas) were also recorded.

Assignment of Patients to Subgroups

As shown in Figure 1 the assignment of patients into hereditary or sporadic melanoma subgroups was done according

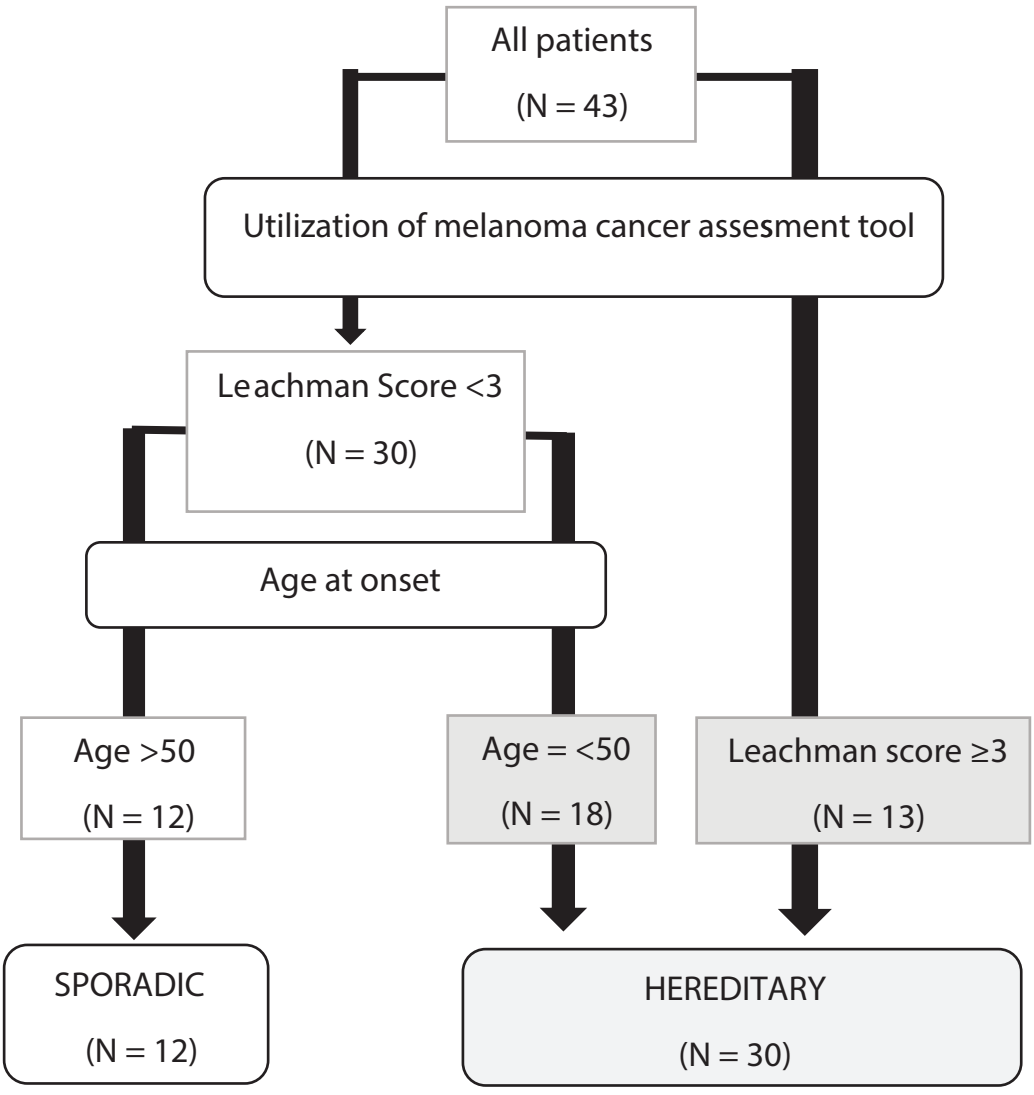

Figure 1. Assignment of melanoma patients into sporadic and hereditary subgroups.

Table 1. Details of Leachman scores of 13 patients who received ≥3 points with the utilization of Melanoma Cancer Syndrome Assessment Tool.

Patient number	Cancer type	Occurrence in the first or second degree relative	Leachman Score
1	Melanoma Breast cancer Prostate cancer	Mother, maternal uncle, Melanoma proband, aunt and sister Father and paternal uncle	6.5
2	Pancreatic cancer Renal cell carcinoma	Paternal grandfather Father	4.5
3	Melanoma Colon cancer	Paternal uncle Daughter	4
4	Breast cancer Colon cancer	2 sisters Maternal uncle	3.5
5	Breast cancer Over cancer	Sister Melanoma proband	3.5
6	Breast cancer Prostate cancer	Aunt Paternal uncle uncle and grandfather	3.5
7	Pancreatic cancer	Aunt	3
8	Pancreatic cancer	Sister	3
9	Pancreatic cancer	Brother	3
10	Second melanoma Pancreatic cancer	Melanoma proband Father	3
11	Melanoma	Sister	3
12	Melanoma	Sister	3
13	Renal cell carcinoma	Father	3

to the "melanoma cancer syndrome assessment tool" [8]. As shown in Table 1 according to this tool 13 patients received ≥3 points (Leachman score ≥3) and were assigned to the hereditary melanoma group. In addition, in this study patients who received <3 points, but who were diagnosed before the age of 50 were also assigned to the hereditary melanoma group. Sporadic melanoma group consisted of 12 patients who scored less than 3 points and who were diagnosed after age 50.

CDKN2A and MC1R germline gene mutations were detected with Sanger sequencing method in laboratories of Hacettepe University Faculty of Medicine, Department of Medical Genetics.

DNA Sequence Analysis with Sanger Method

Polymerase chain reaction (PCR) was performed, including the exon and exon-intron junction points of CDKN2A and MC1R genes. Primer sequences required for amplification were designeed using the PerlPrimer program. GoTaq® (Thermus aquaticus) DNA polymerease enzyme (Promega) was used for the PCR reaction. The PCR reaction was completed under the amplification conditions specified in the Veriti Thermal Cycler device (Thermo Fischer Scientific), and the products were checked. Following the amplification, the purification of PCR products was performed with the QIAquick PCR Purification Kit (Qiagen GmbH) according to the manufacturer protocol. For the sequencing of the PCR products, the sequence reaction consisting of purified PCR product, distilled water, primer and BigDye® terminator mixture was prepared and allowed to react in the thermal cycler. For the sequencing reaction of PCR products forward and reverse primers were used when necessary. Finally, after pre-sequence purification with ZR® DNA Sequencing Clean-up Kit (Zyno Research), samples were ABI 3500 Genetic Analyzer (Applied Biosystems) and analyzed using Sequencing Analysis Software.

Statistics

SPSS 23.0 (IBM) was used to analyze the data. Descriptive statistics were calculated with data. The accordance of the quantitative data for normal distribution was evaluated by the Shapiro-Wilk test. The difference between the two groups of normally distributed independent variables was compared with the significance test of the difference between the two means. The variables not showing normal distribution were compared with Mann-Whitney U test. Qualitative variables were evaluated by Chi-Square test by cross tables. All analyses were tested at the 0.05 significance level.

Results

Patients and Melanoma Characteristics

Twenty-seven (62.6%) of 43 patients were female and 16 (37.2%) were male. The mean age of patients was 50.72±12.21 years (22-75 years). The mean age at diagnosis was 48.95±12.13 years (range 22-70 years). There were 10 patients diagnosed before the age of 40. Of 10 patients diagnosed before age 40, 7 were female and 3 were male. There were 22 patients diagnosed before the age of 50.

Regarding the phenotypic features, the only phenotypic feature that differed in the hereditary and sporadic melanoma groups was the fair eye color, which was more common in the sporadic melanoma group (P = 0.000). All 12 patients (12/12) assigned in the sporadic melanoma group had fair eye color. Whereas 13 out of 31 patients assigned to the hereditary melanoma group had fair eye color.

All other sociodemographic data, features related to sun exposure and clinical characteristics questioned and/or examined in this study displayed similar distributions namely; gender (p = 0.092), hair color (P = 1.000), skin phototype (P = 0.091), presence of freckles (P = 0.719), number of nevi (P = 0.565), number of nevi in the head and neck region (P = 0.208), number of nevi in the upper extremity (P = 0.342), number of nevi on the trunk (P = 0.690), number of nevi in the lower extremity (P = 0.650), number of atypical nevi (P = 0.837), number of lesions excised from the skin (P = 0.316), presence of solar lentigines (P = 0.507), presence of cherry angioma (P = 1.000), ≥1 pigmented lesions in the iris (P = 0.052), history of working outdoors (P = 0.453), outdoor-working time (P = 1.000), participating in outdoor sports and/or similar activities (P = 1.000), history of summer vacation in sunny resorts (P = 0.672), time spent on summer vacation in sunny resorts (P = 0.497), sunbathing (P = 1.000), second degree sunburn (P = 0.497), protection with clothing (P = 0.311) and using sunscreen (P = 0.159).

The histopathological features of melanoma were not statistically different in both group in terms of Breslow thickness (P = 1.000), presence of ulceration (P = 1.000), presence of mitosis (P = 1.000), lymphocytic infiltration (P = 0.721), late regression (P = 1.000) and/or sentinel lymph node biopsy (P = 1.000).

Mutational Analysis

CDKN2A mutation was detected in only 1 patient in the hereditary melanoma group (1/31); whereas no patient in the sporadic melanoma group (0/12) had CDKN2A mutation. The only CDKN2A mutation positive patient belonged to the Leachman score ≥3 subgroup of hereditary melanoma (1/13). As shown in Figure 2 the mutation found was CDKN2A heterozygous A118V.

MC1R mutations were found in 25 out of 31 (80.6%) patients in the hereditary melanoma group; whereas 5 out of 12 (41.7%) patients in the sporadic melanoma group (P = 0.024) (odds ratio=5.833). Among the 13 patients with Leachman score ≥3, 12 had MC1R mutations (92.3%) whereas, 13 out of 18 patients (72.2%) who scored <3 points but were diagnosed before 50 years of age had MC1R mutations.

The most common MC1R variant in all patients was the V60L variant. The distribution of different variants of MC1R mutations observed in hereditary and sporadic

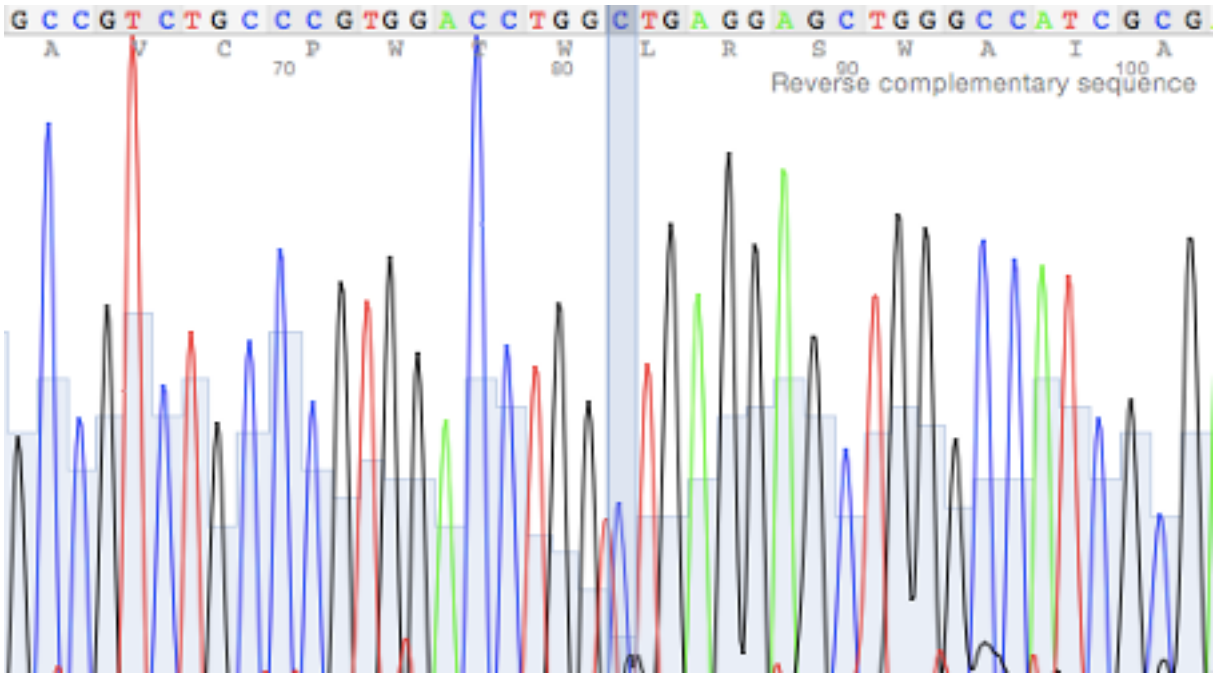

Figure 2. Heterozygous CDKN2A c.353C>T p.A118V. mutation

Table 2. MC1R gene variants in hereditary and sporadic melanoma patients.

MC1R gene variants		Hereditary Melanoma N=31 (%)		
RHC variants	N (%)	Leachman Score ≥3 (N=13) (%)	<50 years of age (N=18) (%)	Sporadic melanoma (N=12) (%)
R160W	7 (16.7)	4 (23.5)	1 (5)	2 (40)
R151C	3 (7.1)	-	2 (10)	1 (20)
R142H	2 (4.8)	1 (5.9)	1 (5)	-
Non-RHC variants				
V60L	9 (21.4)	5 (29.4)	3 (15)	1 (20)
R163Q	8 (19)	3 (17.6)	5 (25)	-
c.942A>G	5 (11.9)	2 (11.8)	3 (15)	-
K278E	2 (4.8)	1 (5.9)	1 (5)	-
C35Y	1 (2.4)	1 (5.9)	-	-
V59L	1 (2.4)	-	1 (5)	-
R67Q	1 (2.4)	-	1 (5)	-
V92M	1 (2.4)	-	1 (5)	-
I120T	1 (2.4)	-	-	1 (20)
c.699A>G	1 (2.4)	-	1 (5)	-

melanoma groups did not show a statistically significant difference. MC1R gene variants, red hair color (RHC) and non- red hair color (non-RHC) variants are listed in Table 2.

Conclusions

The aim of this study was to determine the phenotypic and genetic features that differ among the hereditary and sporadic melanoma groups. In regard to the phenotypic features, results show that fair eye color was the only statistically significantly different feature among all phenotypic features examined in this study. All 12 patients assigned to the sporadic melanoma group in this study had fair eye color, namely blue, green or hazel. In accordance with this finding and taking into consideration that a greater percentage of our population has dark eye color, we suggest that fair eye color should be the alarming sign for dermatologist for a detailed whole body skin examination and a detailed explanation of sun protection to these patients.

CDKN2A mutations were found in 40% of melanoma families worldwide [4]. However, this rate varies geographically. For example, this rate was 20% in Australia where melanoma is very common in contrast, it was 45% in North America and 57% in Europe [11]. Regarding the Mediterranean area in Europe CDKN2A mutations were found in 7.2% of melanoma families in North-eastern Italy and 8.3% for Italy in general [12,13]. In Spain, a total of 30% of the melanoma kindred studied were carriers of CDKN2A variant [14]. In another study conducted in Spain CDKN2A mutation was found in 15 of 87 families (17.2%) [15]. In our study, CDKN2A mutation was detected in only 1 out of 43 patients. This 1 patient belonged to the hereditary melanoma group with Leachman score ≥3 points (N=13). Although the sample size in our study is small, the incidence of CDKN2A in the hereditary melanoma group with Leachman score ≥3 was 1/13 (7.7%), which is in accordance with rates recorded from Italy.

Unfortunately, hereditary melanoma definition is not straight forward and differs from study to study. Because melanoma incidence is quite low in our country, we have decided to keep the criteria for the "hereditary melanoma" as wide as possible in the current study in order not to miss any patients with relevant clues for a hereditary disease. Typical features of true hereditary melanoma/cancer syndromes include features such as unilateral lineage, multi-generational inheritance, multiple primary lesions and early onset of disease [3]. Patients who exhibit all of these features are in fact quite rare. A score ≥ 3 in the Leachman scoring system helps to differentiate patients with predisposition for hereditary cancers. However, age is not used as a criterion in Leachman scoring system and we believe "early age at diagnosis" is a very strong predictor for any disease that is hereditary. This was the reason for a second category in our study as "Leachman score <3 and age at onset <50 years".

Our results with MC1R genetic testing confirm that adding "age" had an impact on the differentiation between the sporadic and the hereditary melanoma patients. As shown in Table 2, MC1R variant positivity was highest in "Leachman score ≥ 3" subgroup of patients, intermediate in the early age at onset subgroup of hereditary melanoma patients and

lowest in the patients assigned for sporadic melanoma group and the difference between the sporadic and hereditary groups were statistically significant. The difference between the two groups in terms of MC1R mutations supports the idea that MC1R genetic testing might help to determine patients with higher risk for hereditary melanoma.

In our patient group the presence of MC1R variants was not associated with hair colour, eye color, skin phototype, presence of solar lentigines, presence of freckles or the number of nevi. This data is in agreement with the fact that most of our patients had dark hair (85.7%) and approximately 50% had skin phototype III and above. This finding strengthens our hypothesis that genetic features may account for the majority of melanomas in our study group rather than phenotypic features or environmental factors.

Studies have reported that MC1R mutations increase the risk of developing melanoma 2-4 times in individuals with both familial and sporadic melanoma [12,16]. It is also reported that this risk was particularly high in individuals with darker skin colour and/or a lower number of nevi [12]. We know that one person can have more than one MC1R variant. To date more than 80 MC1R variants have been identified in Caucasians [6]. The variants and the frequencies of the variants monitored vary according to the geographical regions and prevalence of melanoma. As the variant number of MC1R increases, the risk of new melanoma development increases [12]. It has been reported that the most common variant among MC1R variant in Caucasians is V60L [12,13,16–20]. In our study, in accordance with the literature, the most common variant was also V60L. Our data is consistent with the current literature.

In conclusion, in Turkey, there was no previous data on the CDKN2A and MC1R germline gene mutations in individuals with neither hereditary nor sporadic melanoma. Our study gives at least an estimation of the occurrence of CDKN2A and MC1R germline gene mutations in melanoma patients in Turkey. Located at the capital city our hospital drains patients from all over Turkey and has a wide range of different patient profiles. The small sample size of this study may also be a reflection of the low incidence of melanoma in our country. For those countries where melanoma is rare and where the majority of the population has dark skin, we suggest using "early age at onset" as an additional criterion to melanoma cancer syndrome assessment tool for a more precise detection of hereditary cancer/melanoma cases.

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