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A Melanocortin-4 Receptor Agonist Induces Skin and Hair Pigmentation in Patients with Monogenic Mutations in the Leptin-Melanocortin Pathway

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Keywords

Proopiomelanocortin · Leptin receptor · Melanocortin-4 receptor agonist · Hyperpigmentation · Melanocortin-1 receptor · Leptin-melanocortin pathway

Abstract

Background and Objectives: Gene mutations within the leptin-melanocortin signaling pathway lead to severe early-onset obesity. Recently, a phase 2 trial evaluated new pharmacological treatment options with the MC4R agonist *setmelanotide* in patients with mutations in the genes encoding proopiomelanocortin (POMC) and leptin receptor (LEPR). During treatment with *setmelanotide,* changes in skin pigmentation were observed, probably due to off-target effects on the closely related melanocortin 1 receptor (MC1R). Here, we describe in detail the findings of dermatological examinations and measurements of skin pigmentation during this treatment over time and discuss the impact of these changes on patient safety. ***Methods:*** In an investigator-initiated, phase 2, open-label pilot study, 2 patients

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with loss-of-function POMC gene mutations and 3 patients with loss-of-function variants in LEPR were treated with the MC4R agonist *setmelanotide*. Dermatological examination, dermoscopy, whole body photographic documentation, and spectrophotometric measurements were performed at screening visit and approximately every 3 months during the course of the study. ***Results:*** We report the results of a maximum treatment duration of 46 months. Skin pigmentation increased in all treated patients, as confirmed by spectrophotometry. During continuous treatment, the current results indicate that elevated tanning intensity levels may stabilize over time. Lips and nevi also darkened. In red-haired study participants, hair color changed to brown after initiation of *setmelanotide* treatment. ***Discussion:*** *Setmelanotide* treatment leads to skin tanning and occasionally hair color darkening in both POMC- and LEPR-deficient patients. No malignant skin changes were observed in the patients of this study. However, the results highlight the importance of regular skin examinations before and during MC4R agonist treatment.

Introduction

Proopiomelanocortin (POMC), embedded in the leptin-melanocortin pathway, is cleaved by prohormone convertases into ACTH and α-, β-, and γ-melanocyte-stimulating hormones (MSH). These POMC-derived peptides are capable of activating different types of G protein-coupled melanocortin receptors (MC1R–MC5R). The melanocortin-1 receptor (MC1R), mainly expressed in melanocytes, plays an important role in the regulation of melanin synthesis in the skin, melanocyte differentiation, and DNA repair [1]. The production of eumelanin (dark brown pigment) is of especial importance for protecting the skin against genotoxic UV radiation, as it absorbs UV photons after entering the epidermis [2, 3]. Elevated eumelanin skin concentrations and thereby darker skin color correlate with reduced prevalence of skin cancers like melanoma [1, 4]. In patients with mutations in the POMC gene, the production of POMC derivatives is affected. This impaired α-/β-MSH production leads to missing MC1R activation and thereby to reduced conversion from pheomelanin to eumelanin. This in turn leads to a distinct clinical presentation with pale skin and red hair in several POMC-deficient patients [5–7]. In contrast, patients with leptin receptor (LEPR) deficiency do not lack MSH and generally do not manifest pale skin or red hair secondary to this genetic defect.

The melanocortin system also plays an important role in body weight regulation [5, 8]. POMC-derived peptides activate the MC4R within the hypothalamus, in addition to the MC1R. Impaired function of this leptin-melanocortin signaling leads to severe hyperphagia due to missing activation or alteration of MC4R function. Because of persistent increased hunger feeling, the affected patients are not able to reduce their body weight by traditional conservative treatment strategies (increased exercise and reduced caloric intake). Therefore, a new pharmacological treatment option with the MC4R agonist setmelanotide was evaluated in clinical trials to reduce body weight in patients with impaired function of the leptin-melanocortin pathway due to mutations in the genes *POMC* and *LEPR* (coding for the leptin receptor) [9, 10]. However, setmelanotide activates to a lesser extent the closely related MC1R as an "off-target" effect (MC1R [EC50 effective concentration] = 5.8 nM), as compared to the expected MC4R activation (EC_{50} = 0.27 nM) [9, 10]. This is the reason why setmelanotide treatment affects MC1R-regulated skin and hair color [11, 12]. MC4R is expressed in very low levels in the skin but does not seem to play a fundamental role in pigmentation according to current literature data [13]. The aim of this analysis was to evaluate skin pigmentation using objective measurements in patients with loss-of-function mutations in the POMC and LEPR genes during the treatment with the MC4R agonist *setmelanotide*.

Methods

Study Design and Participants

An investigator-initiated phase 2, nonrandomized, open-label pilot study (EudraCT No. 2014-002392-28; ClinicalTrials.gov No. NCT02507492) with MC4R agonist setmelanotide in severely obese patients with POMC and LEPR mutations was designed and conducted at the Institute for Experimental Pediatric Endocrinology, Charité-Universitätsmedizin Berlin. The study was implemented in cooperation with Rhythm Pharmaceuticals, which provided the study medication and regulatory support [10]. The study was approved by the Ethics Committee of the federal state of Berlin (14/0344), conducted according to the principles of the Declaration of Helsinki, and patients and responsible caretakers gave written informed consent.

Study Medication

Setmelanotide or RM-493 is a synthetic 8-amino acid cyclic peptide, binding with high affinity to MC4R (EC_{50} = 0.27 nM) and less affinity to MC1R (EC_{50} = 5.8 nM) [9, 10]. Setmelanotide treatment was started with a dosage of 0.25–0.5 mg s.c./q.d, followed by a dosage escalation phase up to a maximum concentration of 3 mg s.c./q.d. The maximum dosage administered to each patient depended on weight loss rate and hunger score reduction.

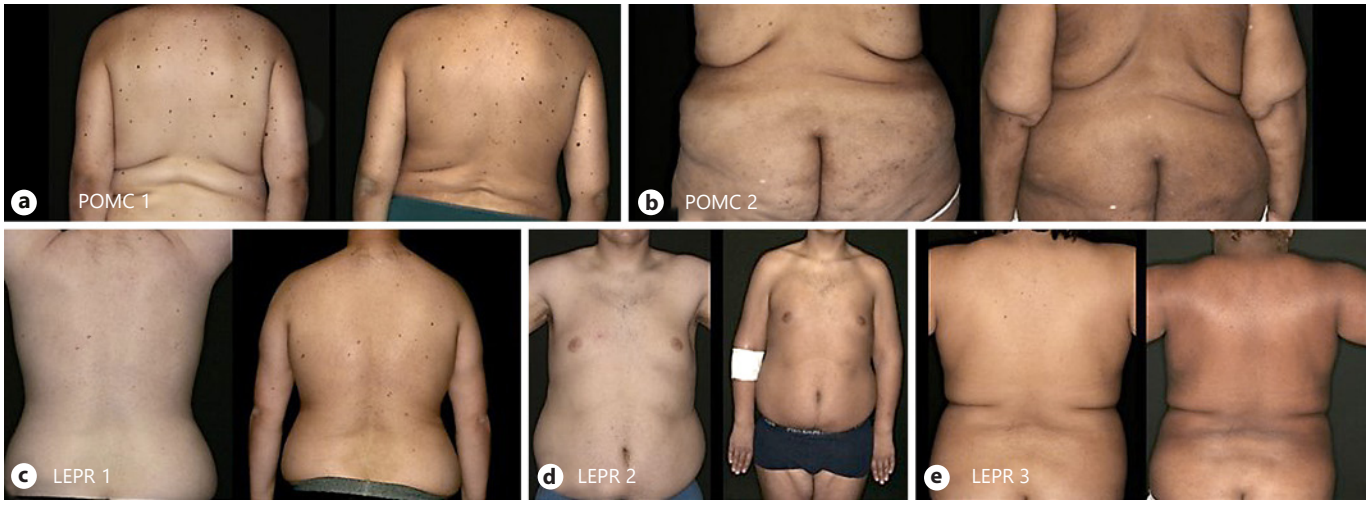

Fig. 1. a–e Comparison of skin color before and during setmelanotide treatment visualizes darkening of the skin of the study patients. POMC, patients with mutations in the gene encoding proopiomelanocortin; LEPR, patients with leptin receptor gene mutations.

Table 1. Summary of patient baseline characteristics including genetic variant and anthropometric data

Patient ID	Gene variant	Sex	Age, years	Weight, kg	BMI, kg/m^2	Fitzpatrick skin type	Hair color	Max. setmelanotide dosage, mg	Origin
POMC 1	Compound heterozygous (G7013T, C7133Δ)	Female	21	155.0	49.8	I	Red/ashy blond	1.5	Caucasian (Northern Europe)
POMC 2	Homozygous (6922InsC)	Female	26	152.8	54.1	II–III	Red/light brown	1.5	African (North Africa)
LEPR 1	Homozygous (p.H684P)	Male	22	122.1	40.7	I	Red	3.0	Caucasian (Northern Europe)
LEPR 2	Homozygous (c.2357T>C p.L786P)	Male	23	130.6	39.9	II–III	Dark brown	1.5	Caucasian (Southern Europe)
LEPR 3	Homozygous (c.2357T>C p.L786P)	Female	14	120.6	44.2	III	Dark brown	2.5	Caucasian (Southern Europe)

Dermatological Examination Procedures

The dermatological examinations were performed at the Department of Dermatology and Allergy, Clinical Research Center for Hair and Skin Science (Charité-Universitätsmedizin Berlin) including dermatological examination, dermoscopy, whole body photographic documentation, and spectrophotometric measurements. These examinations were performed at baseline/screening visit and every 3 months during the course of the study. Determination of skin pigmentation by spectrophotometry was performed in non-UV-exposed axillary and gluteal regions, using the Multi Probe Adapter System MPA® (Courage & Khazaka GmbH, Cologne, Germany) connected to Mexameter® MX18 (Courage & Khazaka Cologne, Germany) as well as the Spectrophotometer CM 700d (Konica Minolta Inc., Tokyo, Japan) according to standardized protocols. The Mexameter® MX18 spectrophotometer emits light of 3 defined wavelengths (green: 568 nm, red: 660 nm, and infrared: 880 nm), and a receiver measures the light reflected by the skin. The results correspond to the spectral absorption peaks of melanin and hemoglobin (as a proxy for erythema), providing melanin and erythema index on a scale from 0 (white) to 999 (black) [14, 15].

The Spectrophotometer CM-700d can measure reflection intensities at a 10-nm wavelength pitch from 400 to 700 nm; the results are presented using the Commission International d' Eclairage L × a × b color system [15, 16]. The L* parameter expresses color brightness, ranging between 0 (white) and 100 (black); the a* parameter expresses changes along a red (+60) to green (−60) axis; the b* parameter represents changes along a yellow (+60) to blue (−60) axis [15]. Both devices are widely used for objective color measurements in skin research [14–22]. Increased melanin indices correlate with increased tanning of the skin [14–22].

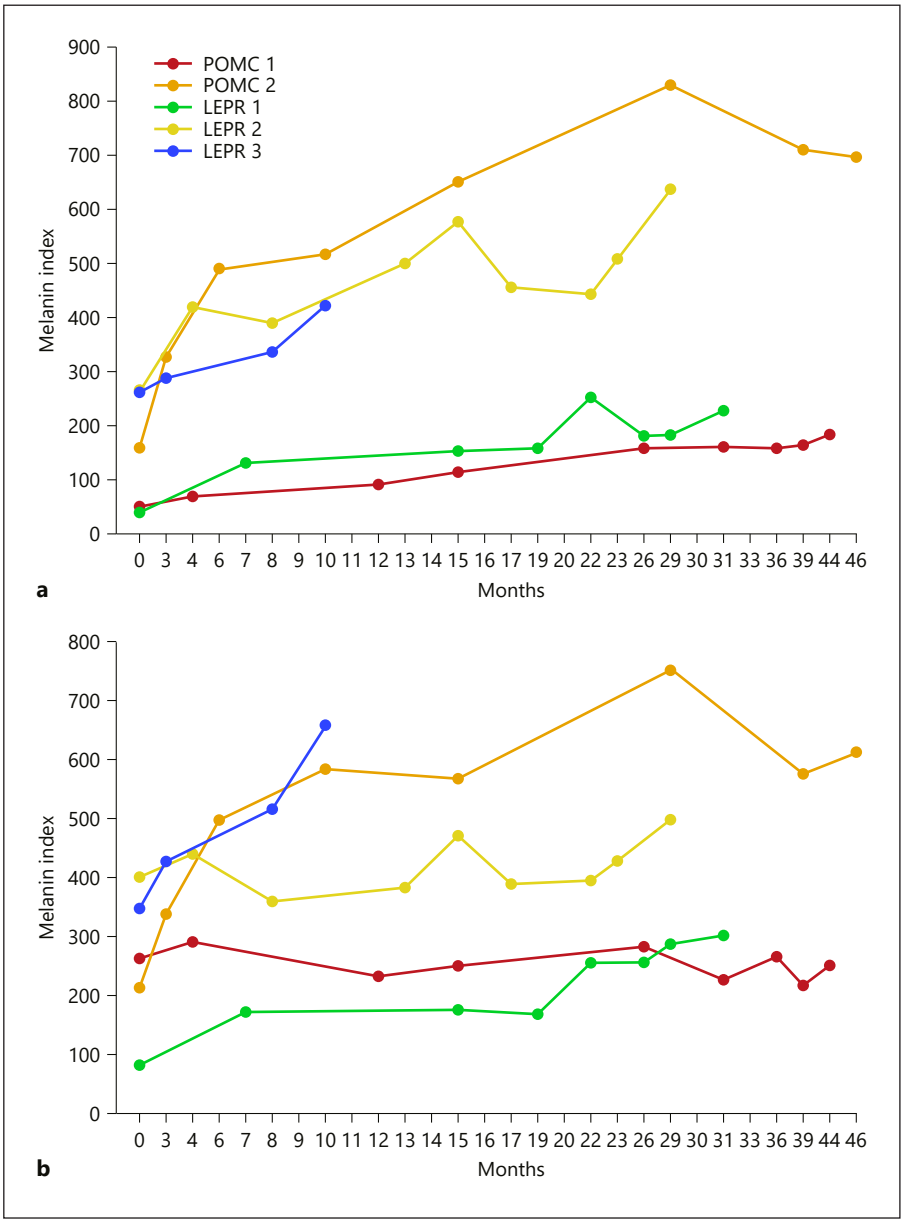

Fig. 2. Spectrophotometry results (melanin index) at baseline and during the course of treatment with setmelanotide in all patients of the study in the gluteal (**a**) and axillary (**b**) region. POMC, patients with mutations in the gene encoding proopiomelanocortin; LEPR, patients with leptin receptor gene mutations.

Statistical Methods

Melanin index values, recorded by Mexameter® MX18, and L* and b* values recorded by the Spectrophotometer CM 700d during the course of the treatments were analyzed in triplicate. Statistical analysis was performed based on nonparametric Student *t* tests. Maximum time of longitudinal observation was 46 weeks. Maximum setmelanotide treatment dosages of each patient are shown in Table 1.

Since we aimed to investigate the effect of *setmelanotide* on melanin synthesis, values expressing erythema (erythema index as measured by using Mexameter® MX18 and a* parameter as measured by using Spectrophotometer CM 700d) are not presented but are available upon request [16]. To evaluate skin color changes over time (slope), difference of melanin raw data between 2 time points was analyzed and divided through the interval (months) between each time point pair. Cases were rated by 0.5 (left and right per time point per body location [axilla/gluteal] in 1 individual). The arithmetic means (±SD) or percentages were reported. No statistical tests were conducted due to the small number of cases ($n = 5$ [1-year follow-up]; $n = 2$ [over 3-year follow-up]).

Statistical analyses were conducted with IBM SPSS 25. The regression coefficients (β) for skin color (melanin index) were generated in a linear mixed-effects regression model. These models have the advantage of dealing with missing values because of using all available data points from an individual longitudinally. Random statement was included to account for the initial differences between individuals. Linear mixed model (random intercept model) [23] was calculated regarding skin color alterations over time with

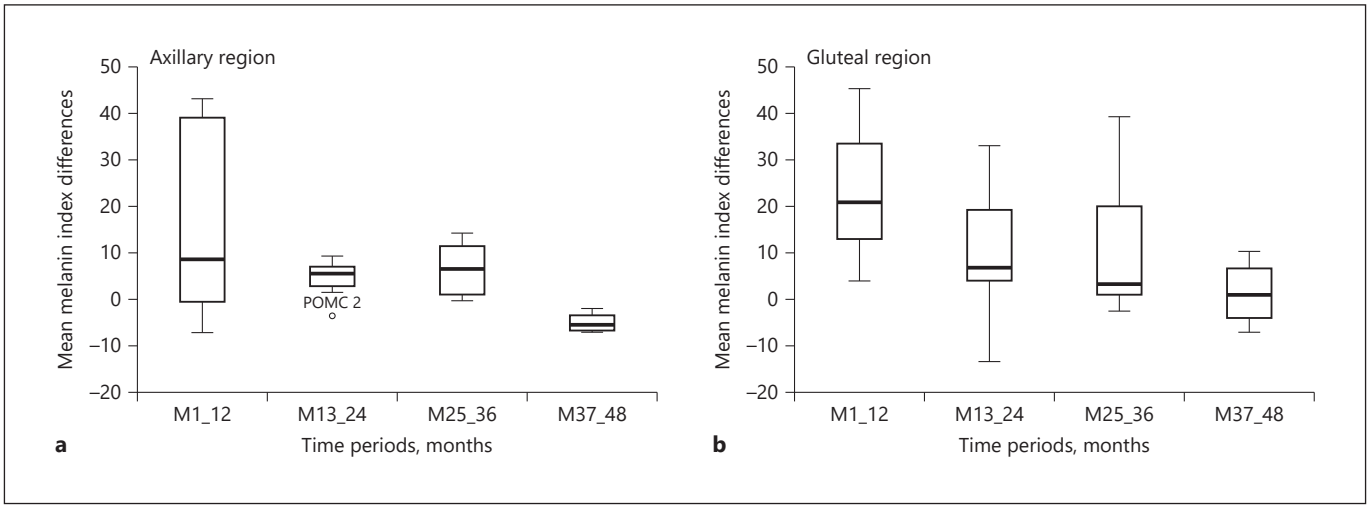

Fig. 3. Slope calculation of melanin values at 4 different time spans of all included patients: treatment months 1–12; treatment months 13–24; treatment months 25–36; treatment months 37–48. **a** Axillary region. **b** Gluteal region.

repeated measurements as level-one units nested in individuals who were level-two units. The model includes adjustment for the localization, where skin color has been measured. For the melanin index variables, linear and quadratic terms for time points were entered into the model in order to describe the skin color curve throughout the different time phases. Two-sided significance level of $\alpha = 0.05$ was used. No adjustment for multiple testing was applied.

Results

In total, 5 patients with monogenic defects within the leptin-melanocortin pathway were included in the study from December 2014 through May 2018: 2 patients with biallelic mutations in the POMC gene and 3 patients with biallelic mutations in the LEPR gene (Table 1). All patients had a history of long-term weight gain due to severe hyperphagia without managing to stabilize body weight using conservative methods for a longer period of time.

Skin and Hair Color Changes

In all patients, skin hyperpigmentation occurred after the treatment start with the MC4R agonist *setmelanotide* (Fig. 1). Accordingly, the L* color parameter decreased and the b* parameter increased during the first months of treatment, reaching a plateau afterwards, with the final values varying interindividually (Spectrophotometer CM 700d, see online suppl. Fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000516282).

Melanin index, measured using Mexameter MX18, increased in all patients during the study, with patients with an African or Southern European background reaching the highest melanin indices (Fig. 2). When comparing the slope of the individual melanin indices, the most prominent increase appears to have occurred within the first few months of treatment. Thereafter, the gradient reduced, which suggests melanin indices reaching a plateau phase (Fig. 2, 3).

Longitudinal analysis with a mixed model confirmed this observation, as it showed a statistically significant association between skin pigmentation and time, adjusted for the localization of the measurement: melanin index increased over the linear term of time (β: 13.15, $p < 0.001$), while there was a statistically significant negative regression coefficient over the quadratic term of time (β: −0.21, $p < 0.001$) [23, 24]. The linear term has more influence on smaller time spans, whereas the quadratic term gains influence on the long term. This indicates a high increase in skin pigmentation for early time points, followed by a stabilization (plateau) during the long-term course of the treatment (online suppl. Fig. 2; online suppl. Table 1).

Also, lips and nevi darkened (Fig. 4) [10, 12]. POMC patient 1 had a history of dysplastic nevus syndrome. During the treatment, 8 nevi were excised due to enhanced pigmentation: 3 showed histological signs of dysplasia but none showed histological signs for malignancy. A further nevus excision was performed in LEPR patient 1 and showed histologically no signs for dysplasia. Regular follow-up of these patients did not show further atyp-

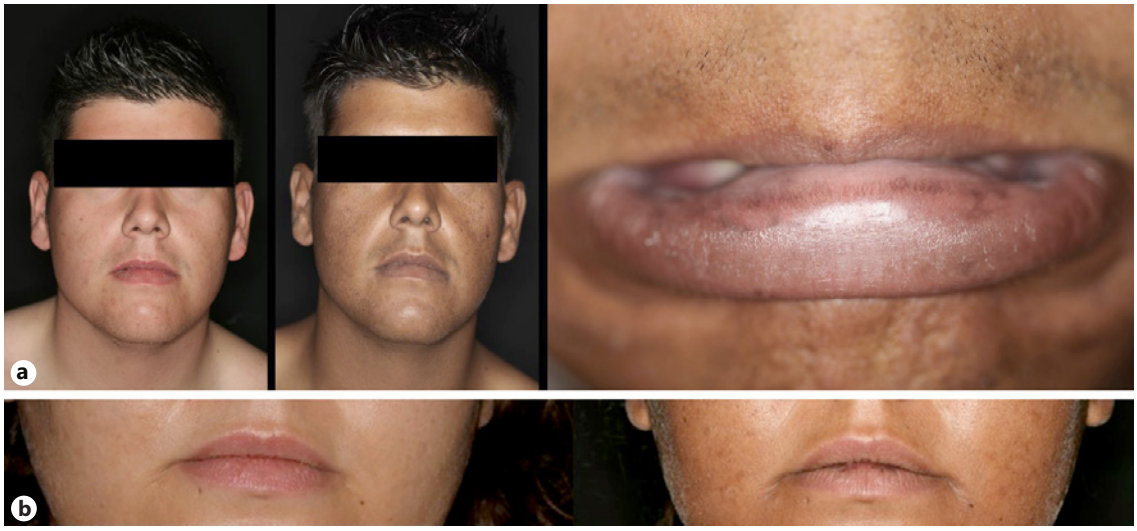

Fig. 4. Intensified pigmentation of the lips in 2 (**a**, **b**) of the study patients during the treatment with setmelanotide.

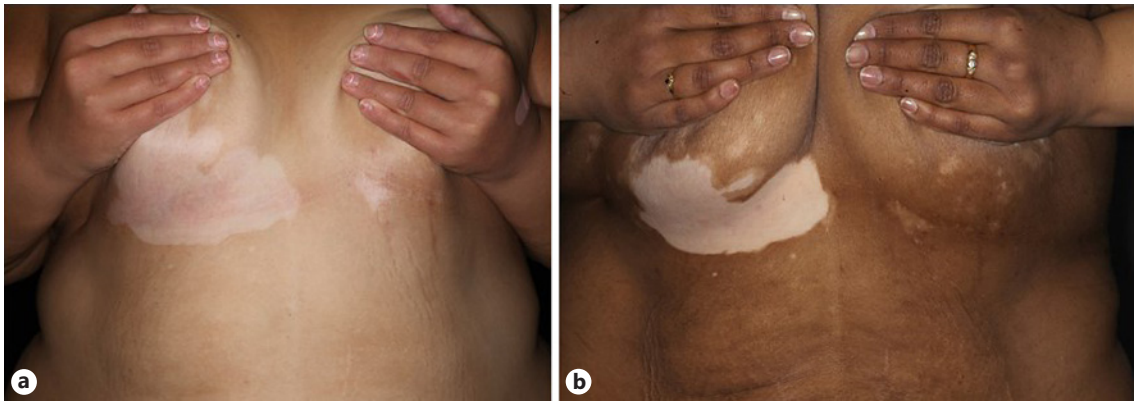

Fig. 5. Partial repigmentation of vitiligo lesions in an enrolled POMC-deficient patient during treatment with *setmelanotide*. Baseline (**a**) and approximately 6 months after treatment initiation (**b**).

ical nevi development under continued treatment with *setmelanotide*. In addition, the other 3 patients did not show any lesions that were suspicious clinically or in dermatoscopy analysis and led to excision.

In POMC patient 2, initially existing vitiligo lesions partially repigmented (Fig. 1b, 5). In initially red-haired patients with either POMC or LEPR deficiency, hair color started to show increased pigmentation and intensified in color after initiation of setmelanotide treatment. After approximately 6 months, hair became brown all over the scalp (Fig. 6a–d). Setmelanotide was generally well tolerated, with only mild adverse events noted during the study [11, 12].

Discussion

The aim of the present analysis was to investigate whether and to what extent treatment with the MC4R agonist *setmelanotide* affects skin pigmentation and hair color based on potential MC1R coactivation in POMC- and LEPR-deficient patients. The study cohort consisted of POMC- and LEPR-deficient patients. Patients with POMC mutations suffer from a lack of both MC4R and MC1R activation due to the missing α-/β-MSH ligands in both brain and skin; the lack of MC1R activation in the skin leads to the typical presentation with pale skin and red hair. This phenotype is thereby comparable with in-

Fig. 6. Hair color changes over time from red to a brown during setmelanotide treatment in 2 enrolled POMC-deficient patients (**a**, **b**) and 1 LEPR-deficient patient (**c**). **d** Hair sample from the POMC mutation carrier visualizes the hair color change during the study. Left side represents hair color before the study. Continuously, hair color changed from red to brown during setmelanotide treatment.

dividuals, in which MC1R genetic variants lead to impaired receptor signaling capacity. These MC1R loss-of-function variants are associated with fair skin, impaired tanning reaction after sun light exposure [25], and an increased risk for skin cancers, like cutaneous melanoma [26, 27], due to missing protection against UV radiation damage due to impaired eumelanin production in melanocytes [26, 28–31]. Therefore, it could be speculated that due to lack of MC1R activation, POMC-deficient patients may have increased risk for melanoma development; a corollary to this speculation is that MC1R activation might provide therapeutic benefit. At this point, it has to be noted that, to our knowledge, until now no case of melanoma development in a POMC patient has been reported in the current literature, yet considering the very rare occurrence of POMC deficiency (orphan disease) and the very young age of patients identified so far.

The second study group consisted of LEPR-deficient patients, who have no defect in α-/β-MSH production and MC1R activation; without the typical baseline abnormalities of POMC-deficient patients, it was of interest to review the impact of MC1R activation during *setmelanotide* treatment also in this study group.

Baseline values of melanin indices (Mexameter MX18) as well as of L* and b* parameters (Spectrophotometer CM 700d) of the patients in this study lie within reported ranges in the recent literature [15, 16, 18]. Comparability of published data sets is however limited, due to different or lack of reporting of measurement areas and different ethnic backgrounds as well as partly due to the variety of

used devices for assessment of skin pigmentation [15, 16, 18, 22]. During the treatment period, an increase in melanin index was observed in all patients, irrespective of their underlying genetic defect [15]. Although data are limited, the results of this study provide evidence for stabilization of increased tanning intensity over time under continued stable treatment with *setmelanotide*. Interestingly, tanning was more intense in patients with an African or southern European background, which might argue for a relevant role of the genetic background. Further evaluations in larger cohorts are necessary to confirm this finding.

Furthermore, it has to be added that data from previous experiments showed that interruption of MC4R agonist dosing leads to a reduction of tanning (Rhythm data on file). The continuous treatment with *setmelanotide* also led to darkening of the hair, presumably due to a transition from red/yellow pheomelanin pigment production to brown/black eumelanin pigment production, indicating hair follicle melanocyte activation [32, 33]. Interestingly, this change of hair color was not only observed in the 2 POMC-deficient patients but also in 1 red-haired LEPR-deficient patient, whose initial hair color was not related to MSH deficiency. This finding suggests a stimulation of the hair follicle MC1R by *setmelanotide*, independently of an underlying MSH deficiency. Increased pigmentation of the lips was observed in some of the study patients, suggesting an increase in the melanogenic activity of the melanocytes also of the oral epithelium [34].

Because of the prolonged stimulation of MC1R resulting in skin, hair, and nevi darkening, it is of importance to evaluate whether continuous MC4R agonist treatment could increase the risk for skin cancers like primary melanoma. So far, in the patients of this study, none of the excised clinically suspicious lesions showed any histological signs of malignancy; however, more data from larger cohorts under prolonged *setmelanotide* treatment are currently missing. During prolonged treatment with *setmelanotide,* regular dermatologic examinations are necessary, in order to confirm long-term safety of this treatment.

In long-term animal studies, *setmelanotide* did not lead to melanocyte proliferation even at very high dosages, and a limited set of completed carcinogenicity studies have not shown any increased risk for cancer development (Rhythm data on file). Additionally, chronic MC1R activation related to other disorders, like in primary adrenal insufficiency (i.e., Addison's disease) due to increased ACTH levels, has not been reported to be related to increased risk for melanoma or other skin cancer development [35, 36]. Recently, responsible regulators approved 2 drugs, which activate MC1R: afamelanotide and bremelanotide. Afamelanotide was approved as a pharmacological treatment of erythropoietic protoporphyria and bremelanotide as a treatment for hypoactive sexual desire disorder [37, 38]. Both drugs may induce pigmentation, depending on the applied formulation, dosage, and frequency of application [37, 38]. For both drugs, no safety concerns regarding the development of skin cancer have been reported so far [37].

Moreover, contrary to the effects of *setmelanotide,* there is evidence based on the available published literature that *reduced* MC1R activation, with less eumelanin and impaired protection against DNA damaging UV radiation, could lead to a predisposition for melanoma, as in patients with loss-of-function mutation in the MC1R gene [2–4]. This hypothesis needs to be tested in larger trials.

Results of this analysis indicate that *setmelanotide* treatment leads to skin tanning in both POMC- and LEPR-deficient patients. Future studies and cautious baseline dermatological examination as well as dermatological monitoring of skin changes during MC4R agonist treatment are necessary to provide further information about the impact of MC4R agonists on skin features. Furthermore, future research could shed more light on possible applications of the observations of this study in dermatological clinical practice, such as in the treatment of depigmentation diseases like vitiligo [13, 39, 40].

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Statement of Ethics

The study was approved by the Ethics Committee of the federal state of Berlin (14/0344), conducted according to the principles of the Declaration of Helsinki, and patients and responsible caretaker gave written informed consent.

Conflict of Interest Statement

Keith Gottesdiener is an employee and stock holder of Rhythm Pharmaceuticals. The remaining authors have no conflicts of interest to declare.

Funding Sources

This is an investigator-initiated trial (sponsor: Charité-Universitätsmedizin Berlin) performed in cooperation with Rhythm Pharmaceuticals, which provided the study medication for the patients as well as financial support for travelling and accommodation of included patients from abroad. The study has been supported by SPARK BIH Validation Fund Track 2.

Author Contributions

P.K. and U.B.P. were responsible for planning and supervising the study. C.P.B., K.C., I.F., S.W., and P.K. were referent physicians for the patients. V.K., I.J., U.B.P., A.A., and C.R. were responsible for the dermatological study performance. V.K., L.P., I.J., and P.K. processed the experimental data, performed the analysis, drafted the manuscript, and designed the figures. V.K. took the lead in writing the manuscript. All authors aided in interpreting the results, reviewed the final manuscript, and approved publication.

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