Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by *POMC* mutations in humans

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Sequential cleavage of the precursor protein pre-pro-opiomelanocortin (POMC) generates the melanocortin peptides adrenocorticotrophin (ACTH), melanocyte-stimulating hormones (MSH) α, β and γ as well as the opioid-receptor ligand β-endorphin[1]. While a few cases of isolated ACTH deficiency have been reported (OMIM 201400), an inherited POMC defect has not been described so far[2]. Recent studies in animal models elucidated a central role of α-MSH in the regulation of food intake by activation of the brain melanocortin-4-receptor (MC4-R; refs 3–5) and the linkage of human obesity to chromosome 2 in close proximity to the *POMC* locus[6], led to the proposal of an association of *POMC* with human obesity[7].The dual role of α-MSH in regulating food intake and influencing hair pigmentation predicts that the phenotype associated with a defect in *POMC* function would include obesity, alteration in pigmentation and ACTH deficiency. The observation of these symptoms in two probands prompted us to search for mutations within their *POMC* genes. Patient 1 was found to be a compound heterozygote for two mutations in exon 3 (G7013T, C7133Δ) which interfere with appropriate synthesis of ACTH and α-MSH. Patient 2 was homozygous for a mutation in exon 2 (C3804A) which abolishes *POMC* translation. These findings represent the first examples of a genetic defect within the *POMC* gene and define a new monogenic endocrine disorder resulting in early-onset obesity, adrenal insufficiency and red hair pigmentation.

Patient 1 (Fig. 1*a*), from family 1, displays obesity, red hair pigmentation and ACTH deficiency. Direct sequencing of PCR products covering the entire *POMC* coding region of members of family 1 revealed two different mutations in exon 3 (Fig. 2). A G→T transversion in the paternal allele at nucleotide position (nt) 7013 results in a premature termination at codon 79 (see refs 8,9 for numbering of genomic and protein sequence). Truncation of the POMC protein at codon 79 predicts complete absence of ACTH, α-MSH and β-endorphin, encoded further downstream (Fig. 2*d*). In the maternal allele, a 1-bp deletion of nt 7133 (Fig. 2*b*) causes a frame-shift predicted to disrupt the structure of the receptor-binding core motif of ACTH and α-MSH (HFRW→HFAG) and introduces a premature termination at codon 131 (Fig. 2*d*). Compound heterozygosity for these two mutations was confirmed in patient 1, the second-born daughter of family 1 (Fig. 1*a*), and in the first-born son who died at the age of seven months of hepatic failure following severe cholestasis, which was, in the postmortem examination, found to be caused by adrenal insufficiency due to bilateral adrenal hypoplasia. Mutational analysis in the son was performed retrospectively in DNA eluted from a stored newborn-screening filter-paper blood specimen. Due to the structural changes introduced by these two mutations, a complete loss of POMC-derived ACTH, α-MSH and β-endorphin in the compound heterozygous patients can be expected. Accordingly, we were not able to detect pituitary-derived POMC peptides in the serum of patient 1, even after stimulation (Table 1). The normal values of all other anterior pituitary-derived hormones exclude developmental defects of the pituitary and hypothalamus.

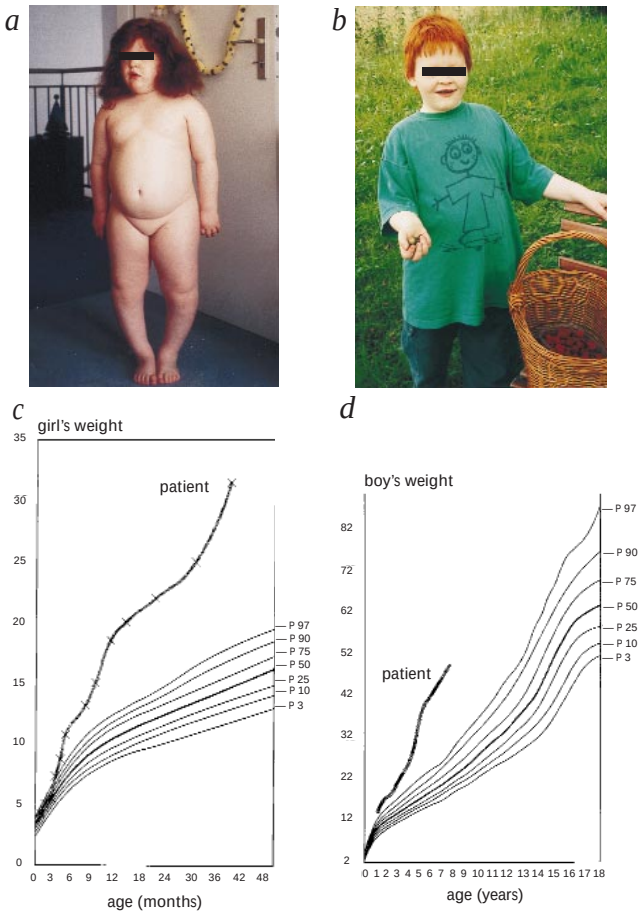

Fig. 1 Phenotype and weight curves of patient 1 and 2. ***a***, Patient 1 is shown at three years of age, demonstrating the red-hair pigmentation and obesity. She had normal weight at birth and developed cholestasis at three weeks. Due to the history of adrenal hypoplasia in her first-born brother, the ACTH deficiency was diagnosed at 23 days and hydrocortisone substitution led to subsequent resolution of cholestasis. Since she was four months of age, the parents reported an increased appetite which led to severe early-onset obesity interfering with the ability to walk until she was two years. Mental development so far has been normal. ***b***, Patient 2 is shown at an age of five years. The perinatal history was complicated by transient hypoglycaemia. His birth weight was normal and obesity was first noticed at five months . After a febrile seizure attack at 12 months, blood-glucose measurement revealed hypoglycaemia and hyponatriaemia leading to an endocrine work-up which resulted in the diagnosis of adrenal insufficiency due to complete ACTH deficiency. With hydrocortisone substitution, his subsequent development was uneventful apart from abnormal eating behaviour causing progredient obesity. His intellectual and emotional assessments (HAWIK test at five years) were normal. In both children, MRT imaging revealed normal pituitary morphology. ***c,d***, Auxology of patients 1 (*c*) and 2 (*d*) demonstrating progredient obesity in both cases. The weight curves of the patients are indicated in red. The photographs are reproduced with the written informed consent of the parents.

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Table 1 • Hormonal levels in patients 1 and 2

	ACTH [pg/ml]	Cortisol [µg/dl]	ACTH [pg/ml]	Cortisol [µg/dl]	LH [mU/l]	FSH [mU/l]	GH [ng/ml]	TSH [µU/ml]	PL [ng/ml]	α-MSH [pmol/l]
	60 min after CRH		peak in profile		60 min after LHRH		60 min after GHRH		30 min after TRH	
patient 1	< 5	< 1	< 5	< 1	8.7	20.2	14.2	*	32	nd
patient 2	< 5	< 1	< 5	< 1	*	*	7.2	12.6	28	15
normal range	> 15	> 18–28	>10	> 10	1.5–4.0	3.7–15.6	> 10	> 8.5–20	> 12.5–45	9.2–100

LH, luteinizing hormone; LHRH, luteinizing hormone-releasing hormone; FSH, follicle-stimulating hormone; GH, growth hormone; GHRH, growth hormone-releasing hormone; TSH, thyroid-stimulating hormone, thyrotropin; TRH, thyrotropin-releasing hormone; PL, prolactin; *, not determined; nd, not detectable.

Sequencing of PCR products of the *POMC* gene of patient 2 (Fig. 1*b*) revealed a homozygous C→A transversion at nt position 3804 in exon 2, located 11 bp upstream of the start codon within the 5′ untranslated region (Fig. 3). This mutation creates an additional out-of-frame ATG initiation codon within a consensus sequence for translation initiation (Fig. 3*d*). According to the current 'scanning model' of eukaryotic translation[10], the introduction of an additional out-of-frame start codon could abolish translation of the wild-type protein, as shown in mutagenesis studies using the preproinsulin gene[11]. Consistent with this scenario, we found only trace amounts of POMC-derived peptides in the patient's serum (Table 1). We failed to detect the C3804A mutation in 50 DNA samples from healthy controls by restriction-endonuclease digestion analysis with *Sph*I (data not shown), indicating that it is not a common polymorphism. Only few mutations which interfere with translation initiation[12,13] have been reported in humans. Although they do not include the description of an additional out-of-frame initiation codon, the pathogenic significance of this kind of mutation has recently been demonstrated in the macaque carbonic anhydrase gene I (ref. 14).

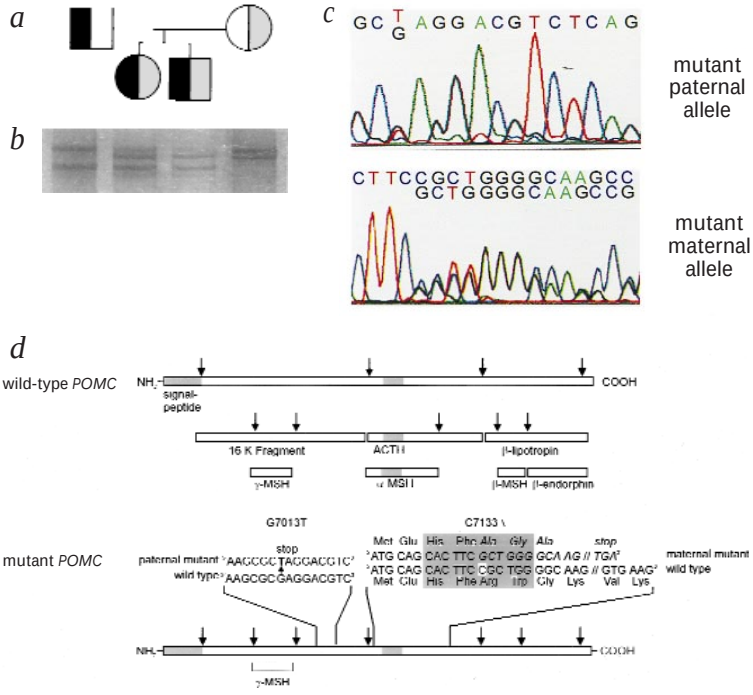

Fig. 2 *POMC* mutation in family 1. ***a***, Pedigree of family 1. Half-filled symbols indicate the heterozygous state of the parents, whereas both children are compound heterozygous. The first-born son died at seven months due to hepatic failure after severe cholestasis which was explained postmortem by the diagnosis of bilateral adrenal atrophy. He also had a seizure correlated with hypoglycaemia at eight weeks. The second-born daughter represents patient 1 of this study. ***b***, SSCP analysis of PCR products of exon 3 of all family members demonstrates compound heterozygosity of both affected children and heterozygosity of both parents. ***c***, Sequencing of exon 3 of *POMC* of patient 1 revealed two compound heterozygous mutations: a G→T change at nt 7013 and a deletion of nt 7133 (sequencing of exon 3 of both parents revealed the heterozygous G→T alteration at nt 7013 in the father and the heterozygous C deletion at nt 7133 in the mother, data not shown). ***d***, Predicted structural consequence of the two mutations within the POMC protein sequence encoded by exon 3 (ref. 8). The cleavage-sites generating the different POMC derived peptides are indicated by arrows above the wild-type protein[1]. Differences in POMC processing resulting from mutations in both alleles of the patient are shown, predicting loss of POMC derived peptides apart from γ-MSH. The altered frame generated by the C deletion at nt 7133 is shown in italics and results in a premature termination at codon 131.

Consistent with our knowledge about the function of POMC-derived peptides, the phenotype of both patients seems to reflect the lack of ligands for the melanocortin receptors MC1-R, MC2-R and MC4-R. The relevance of MC1-R signalling for coat pigmentation has been established in several animal models. While in the agouti mice strain A^y antagonism of overexpressed Agouti protein at the MC1-R causes the characteristic yellow pigmentation[15], a loss-of-function mutation of the *MC1R* gene in the Norwegian[16] and the Holstein[17] cattle results in red coat phenotypes. In humans, the contribution of MC1-R function to hair and skin pigmentation has been proposed on the basis of correlation studies of several *MC1R* polymorphisms with red hair in British and Irish individuals[18] and recently in an Australian study of red-haired twins[19]. The red hair pigmentation in our patients (Fig. 1*a*,*b*) implies that the loss of POMC peptides results in a lack of ligands activating the MC1-R, most likely α-MSH, and argues for the same critical role of MC1-R signalling for human pigmentation. Disruption of MC2-R signalling explains the clinically dominant symptoms due to ACTH-deficiency, that is hypocortisolism and hypoglycaemia as ACTH of pituitary origin is the only known ligand for the adrenal MC2-R (ref. 20). Recent studies in mice revealed the important role of MC4-R signalling in the regulation of energy stores. Intracerebroventricular administration of a MC4-R-selective α-MSH antagonist[5], targeted disruption of the *MC4R* gene[4] as well as antagonism of ectopically overexpressed Agouti peptide at the MC4-R as shown in the agouti obesity syndrome[3], result in stimulation of food intake and severe obesity in mice. Consistent with this role of MC4-R signalling in feeding control in rodents, the severe early-onset obesity seen in our patients can be explained by the absence the MC4-R ligand α-MSH. This would be consistent also with a major contribution of α-MSH in the pathogenesis of obesity in prohormone-convertase-1 deficiency[21] as in this syndrome circulating POMC is increased, presumably reflecting inappropriate processing of α-MSH. Moreover, the relevance of α-MSH rather than γ-MSH for the regulation of food intake is underscored by the normal processing of γ-MSH in patient 2 (Fig. 2*d*), also consistent with the low affinity of γ-MSH for the MC4-R (ref. 20). The normal phenotype of the heterozygous parents in both families (Figs 2*b*,3*b*) suggests a recessive mode of inheritance.

In conclusion, these children represent the first reported cases of a POMC-deficiency syndrome. The variations in hair pigmentation, adrenal function and body weight are consistent with the lack of POMC-

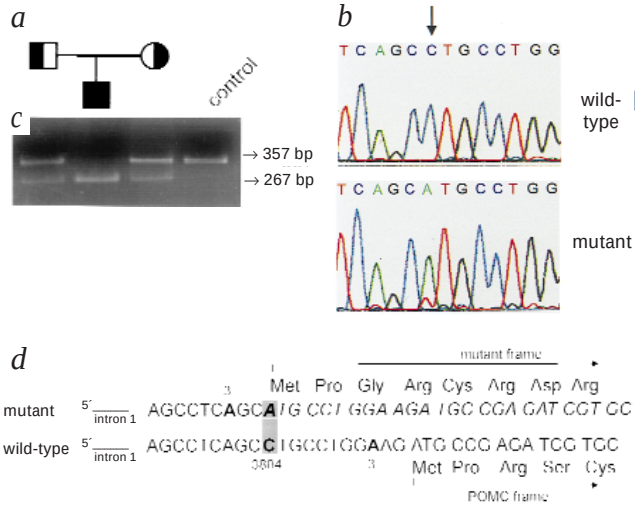

Fig. 3 *POMC* mutation in family 2. ***a***, Pedigree of family 2. The only son born to nonconsanguineous healthy parents represents patient 2 of this study. Half-filled symbols indicate the heterozygous state of the parents and filled symbols the homozygous state of the patient. ***b***, Sequencing of *POMC* exon 2 revealed a homozygous C→A change at nt 3804 in patient 2 (arrow). ***c***, Restriction-enzyme analysis with *Sph*I was performed in all family members and a healthy control. The homozygosity of the patient and heterozygosity of both parents was demonstrated whereas the control sample of exon 2 was not cut by *Sph*I. ***d***, Predicted functional consequences of the C3804A mutation for *POMC* translation is shown according to the scanning model of eukaryotic translation[10] which predicts the exclusive initiation at the first 5´-initiation codon. The A at position –3 which represents the minimal requirement of the consensus sequence for translation initiation is shown in bold. Underlined base pairs which are homologous to the consensus sequence (GCCGCC A/G CCATGG) demonstrate the same degree of homology of the wild-type and mutant start codon.

derived ligands for the MC1-R, MC2-R and MC4-R, respectively. It will be interesting to examine the exocrine secretion in the two patients, as targeted disruption of the MC5-R has revealed a regulatory role of melanocortin peptides for exocrine gland function[22]. The lack of symptoms related to β-endorphin deficiency might be due to the redundancy of ligands in the opioid receptor network. In addition to the genes encoding prohormone-convertase 1 (ref. 21), leptin[23,24] and the leptin receptor[25], for which genetic defects in extreme cases of human obesity have been described, the *POMC* gene represents the fourth candidate gene implicated in the aetiology of human obesity. In the future, the development of MSH-analogues as therapeutic tools to control eating disturbances, at least in particular early onset cases like those presented here, might be considered.

Methods

Assessment of endocrine function. ACTH was determined using a two-sited immunometric assay (Brahms). α-MSH was measured with a commercially available RIS system (IBL). The level of crossreactivity to ACTH 1-13, ACTH 1-14, ACTH 1-39, β- and γ-MSH was less than 0.002%.

Sample preparation and *POMC* analysis. After informed consent, DNA was extracted from peripheral white blood cells of all family members using a DNA extraction kit (QIAamp Blood Kit). For DNA extraction of dried blood spots from newborn screening filter-paper, incubation with Proteinase K in PBS was performed overnight. The coding sequence of *POMC* was PCR-amplified with a 5´-sense primer (5´–GCTCAAGGTCCTTCCTG-GTG–3´) and an 3´-antisense primer (5´–GCCCTGGATTGAATCACGCC–3´) to generate a fragment encompassing exon 2, and with two pairs of primers (5´–CGGCCAGGGCCTAGGCGCAG–3´ and 5´–TCGTCCTCG-GCGCCGTTAGG–3´; 5´–AGCCGGTGGGCAAGAAGCGG–3´ and 5´–CCAGGCTGGGAGGCGGCAGC–3´) to generate overlapping fragments of exon 3. The reaction consisted of 2 min at 94 °C followed by 30 cycles of 30 s at 94 °C, 30 s at 68 °C and 1 min at 72 °C. Direct sequencing of the double-stranded PCR products was carried out from both directions using the ABI PRISM Dye Terminator Cycle Sequencing Kit and an automated fluorescent sequencer (Applied Biosystems). In parallel, single-strand conformational polymorphism (SSCP) analysis from the amplified PCR products was performed as described[26]. Restriction-endonuclease digestion analysis to screen for the C→A transversion at nt 3804 was performed with *Sph*I; products were separated on a 2% agarose gel.

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