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 pro-pre-opiomelanocortin (POMC) generates the melanocortin peptides adrenocorticotropic hormone (ACTH), melanocyte-stimulating hormones (MSH) α, β and γ as well as the opioid-receptor ligand β-endorphin1. While a few cases of isolated ACTH deficiency have been reported (OMIM 201400), an inherited POMC defect has not been described so far2. 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 (MC4R; refs 3–5) and the linkage of human obesity to chromosome 2 in close proximity to the POMC locus6, led to the proposal of an association of POMC with human obesity7. 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 two 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, C7133A) 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. 1a), 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. 2d). In the maternal allele, a 1-bp deletion of nt 7133 (Fig. 2b) causes a frame-shift predicted to disrupt the structure of the receptor-binding core motif of ACTH and α-MSH (HFAG→HFAQ) and introduces a premature termination at codon 131 (Fig. 2d). Compound heterozygosity for these two mutations was confirmed in patient 1, the second-born daughter of family 1 (Fig. 1a), 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. Muta-

tional 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.

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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 measurements revealed hypoglycaemia and hyponatraemia 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 pro-gredient 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 pro-gredient 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.
Sequencing of PCR products of the POMC gene of patient 2 (Fig. 1b) 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. 3d). According to the current 'scanning model' of eukaryotic translation, 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. Consistent with this scenario, we found only trace amounts of POMC C-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 SphI (data not shown), indicating that it is not a common polymorphism. Only few mutations which interfere with translation initiation 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 gene11.12.

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, targeted disruption of the MCR4 gene6 as well as antagonism of ectopically overexpressed Agouti peptide at the MC4-R as shown in the agouti obesity syndrome, 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 of 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-converting enzyme 1 deficiency 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. 2b, 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 2b,3b) 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-

### Table 1 • Hormonal levels in patients 1 and 2

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<td>&gt;10</td>
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<td>3.7-15.6</td>
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<td>&gt;8.5-20</td>
<td>&gt;12.5-45</td>
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LH, luteinizing hormone; LHRH, luteinizing hormone-releasing hormone; FSH, follicle-stimulating hormone; GH, growth hormone; GHRH, growth hormone-releasing hormone; TSH, thyroid-stimulating hormone; TRH, thyrotropin-releasing hormone; PL, prolactin; *, not determined; nd, not detectable.

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 compatible 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, Sequence 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. 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.
shown according to the scanning model of eukaryotic translation which pre-demonstrated whereas the control sample of exon 2 was not cut by SplI. The homozygosity of the patient and heterozygosity of both parents was demonstrated whereas the control sample of exon 2 was not cut by SplI. Pre-diction of functional consequences of the C8384A mutation for POMC translation is shown in the Table 1. Underlined base pairs which are homologous to the consensus sequence (GCCGCCA/A CGATGG) demonstrate the same degree of homology of the wild-type and mutant start codon.

Methods

Assessment of endocrine function. ACTH was determined using a two-stated immunometric assay (Brahms). e-MSH was measured with a commercially available RIS system (IBL). The level of crossreactivity to ACTH 1-39, ACTH 1-14, ACTH 1-39, 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’-GCTCAAGGTCCCTCTGTTG-3’) and an 3’-antisense primer (5’-GCCCCTGATTAGTCACGC-3’) to generate a fragment encompassing exon 2, and with two pairs of primers (5’-CCGCCAGGCTTAGGCAC-3’ and 5’-TCGTCCTCGGCGCCCTAGG-3’) to generate overlapping fragments of exons 1 and 3. The reaction consisted of 30 cycles at 94 °C followed by 30 cycles of 94 °C 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 DYEs 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. Restriction-endonuclease digestion analysis to screen for the C→A transversion at nt 3804 was performed with SplI; products were separated on a 2% agarose gel.

Acknowledgements

The authors thank J. Schwarz for helpful discussions and K. Huhne for excellent technical assistance. This study was supported by a grant from the Deutsche Forschungsgemeinschaft to H.K. (Kr17101/1) and the Sonnenfeld-Stiftung to H.B.

Received 15 April; accepted 5 May, 1998.


