Intracellular Retention of Human Melanocortin-4 Receptor: A Molecular Mechanism Underlying Early-onset Obesity in F261S Pedigree of Chinese[1]

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Objective To investigate how F261S mutation identified from Chinese obese patients affects the function of melanocortin 4 receptor (MC4R) and to analyze the obesity-related phenotypes in subjects carrying the F261S mutation. **Methods** F261S mutant of MC4R was generated by site-directed mutagenesis. Plasmids encoding wild-type or F261S mutant of MC4R were transfected into HEK293 and COS-7 cells to examine their functional characteristics. Signaling properties of F261S MC4R were assessed by measuring intracellular cAMP levels in response to α-MSH stimulation. Cell surface expression of F261S MC4R was compared with that of wild-type MC4R. Clinical examinations were performed in subjects carrying F261S mutation and in non-mutated controls. **Results** The α-MSH-stimulated reporter gene activity was significantly reduced in cells expressing F261S MC4R, with a maximal response equal to 57% of wild-type MC4R. The F261S mutation also led to a significant change in the EC_{50} value compared with the wild-type receptor ($P<0.01$). Immunofluorescent assay revealed a marked reduction in plasma membrane localization of the MC4R in cells expressing the F261S mutant receptor. The resting metabolic rate and fat composition of the mutant carriers were not significantly different from those of the non-mutated obese controls. **Conclusions** The decreased response to α-MSH due to the intracellular retention of MC4R may cause early-onset obesity in the F261S pedigree of Chinese.

Key words: Obesity; Melanocortin 4 receptor; Mutation; Chinese

REFERENCES

1 Hill J O, Peters J C (1998). Environmental contributions to the obesity epidemic. *Science* **280**, 1371-1374.
2 Comuzzie A G, Allison D B (1998). The search for human obesity genes. *Science* **280**, 1374-1377.
3 Barsh G S, Farooqi I S, O'Rahilly S (2000). Genetics of body-weight regulation. *Nature* **404**, 644-651.
4 Snyder E E, Walts B, Perusse L, *et al*. (2004). The human obesity gene map: the 2003 update. *Obes Res* **12**, 369-439.
5 Gantz I, Miwa H, Konda Y, *et al.* (1993). Molecular cloning, expression, and gene localization of a fourth melanocortin receptor. *J Biol Chem* **268**, 15174-15179.
6 Sundaramurthy D, Campbell D A, Leek J P, *et al.* (1998). Assignment of the melanocortin 4 receptor (MC4R) gene to human chromosome band 18q22 by *in situ* hybridisation and radiation hybrid mapping.*Cytogenet Cell Genet* **82**, 97-98.
7 Mountjoy K G, Robbins L S, Mortrud M T, *et al*. (1992). The cloning of a family of genes that encode the melanocortin receptors. *Science* **257**, 1248-1251.
8 Fan W, Boston B A, Kesterson R A, *et al.* (1997). Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature* **385**, 165-168.
9 Ollmann M M, Wilson B D, Yang Y K, *et al.* (1997). Antagonism of central melanocortin receptors *in vitro* and *in vivo* by agouti-related protein. *Science* **278**, 135-138.
10 Vaisse C, Clement K, Guy-Grand B, *et al.* (1998). A frameshift mutation in human MC4R is associated with a dominant form of obesity. *Nat Genet* **20**, 113-114.
11 Yeo G S, Farooqi I S, Aminian S, *et al.* (1998). A frameshift mutation in MC4R associated with dominantly inherited human obesity. *Nat Genet* **20**, 111-112.
12 Tao Y X (2005). Molecular mechanisms of the neural melanocortin receptor dysfunction in severe early onset obesity. *Mol Cell Endocrinol* **239**, 1-14.
13 Kublaoui B M, Zinn A R (2006). Editorial: MC4R mutations--weight before screening! *J Clin Endocrinol Metab*

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91, 1671-1672.
14 Cai S B, Jia W P, Fang Q C, *et al.* (2004). F261SA novel mutation of melanocortin 4 receptor gene in the obese patients. *Chin J Endocrinol Metab* **20**, 372-375. (In Chinese)
15 Gotoda T, Scott J, Aitman T J (1997). Molecular screening of the human melanocortin-4 receptor gene: identification of a missense variant showing no association with obesity, plasma glucose, or insulin. *Diabetologia* **40**, 976-979.
16 Shao X Y, Jia W P, Cai S B, *et al.* (2005). Cloning and functional analysis of melanocortin 4 receptor mutation gene F261S. *Chin Med J* **85**, 366-369 (in Chinese).
17 Group of China Obesity Task Force (2004). Body mass index reference norm for screening overweight and obesity in Chinese children and adolescents. *Chin Epidemial J* **25**, 97-102. (In Chinese).
18 Karhunen L, Franssila-Kallunki A, Rissanen A, *et al*. (1997). Determinants of resting energy expenditure in obese non-diabetic caucasian women. *Int J Obes Relat Metab Disord* **21**, 197-202.
19 Kunkel T A, Roberts J D, Zakour R A (1987). Rapid and efficient site-specific mutagenesis without phenotypic selection. *Methods Enzymol* **154**, 367-382.
20 Marsh D J, Hollopeter G, Huszar D, *et al.* (1999). Response of melanocortin-4 receptor-deficient mice to anorectic and orexigenic peptides. *Nat Genet* **21**, 119-122.
21 Ste Marie L, Miura G I, Marsh D J, *et al.* (2000). A metabolic defect promotes obesity in mice lacking melanocortin-4 receptors. *Proc Natl Acad Sci U S A* **97**, 12339-12344.
22 Butler A A, Marks D L, Fan W, *et al.* (2001). Melanocortin-4 receptor is required for acute homeostatic responses to increased dietary fat. *Nat Neurosci* **4**, 605-611.
23 Rong R, Tao Y X, Cheung B M, *et al.* (2006). Identification and functional characterization of three novel human melanocortin-4 receptor gene variants in an obese Chinese population. *Clin Endocrinol (Oxf)* **65**, 198-205.
24 Yang X, Wang Z, Dong W, *et al.* (2003). Modeling and docking of the three-dimensional structure of the human melanocortin 4 receptor. *J Protein Chem* **22**, 335-344.
25 Tao Y X, Segaloff D L (2003). Functional characterization of melanocortin-4 receptor mutations associated with childhood obesity. *Endocrinology* **144**, 4544-4551.
26 Lubrano-Berthelier C, Durand E, Dubern B, *et al.* (2003). Intracellular retention is a common characteristic of childhood obesity-associated MC4R mutations. *Hum Mol Genet* **12**, 145-153.
27 Farooqi I S, Keogh J M, Yeo G S, *et al.* (2003). Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. *N Engl J Med* **348**, 1085-1095.
28 Farooqi I S, Yeo G S, Keogh J M, *et al.* (2000). Dominant and recessive inheritance of morbid obesity associated with melanocortin 4 receptor deficiency. *J Clin Invest* **106**, 271-279.
29 Mergen M, Mergen H, Ozata M, *et al.* (2001). A novel melanocortin 4 receptor (MC4R) gene mutation associated with morbid obesity. *J Clin Endocrinol Metab* **86**, 3448-3451.

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