

Individual variation in response to μ opiate receptor challenge—past, present, and future: a “personal” history of investigation

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Individual differences in response to addictive substances may provide important clues to the mechanisms underlying drug action, addiction, reward, and reward-related disease states. Early psychoneuroendocrinological studies have led to the distinction of responders and nonresponders upon μ opiate receptor agonist administration. The systematic analysis of the gene encoding the μ opiate receptor reveals abundant DNA sequence diversity, suggesting numerous individually different forms of the gene. The present work illustrates the challenges of establishing complex genotype-phenotype relationships in the presence of high natural sequence variation, and provides some preliminary solutions. Progress in the future is expected to come from whole systems analysis-based approaches, integrating variation in all genes in all pathways.

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The examination of human individual differences at all levels of biological and phenotypic analysis will provide important insights into the mechanisms underlying complex traits. In particular, individual differences in response to addictive substances may help to elucidate the mechanisms underlying drug action, addiction, reward, and reward-related disease states. “The individual” has, both conceptually and concretely, been banned for far too long from approaches to scientific investigation. At the heart of endeavours to describe the functions and dysfunctions of “the” organism was the determination of mean values, as the averages of all individual values, and a standard error that indicated the extent of deviation of the individual values from the “mean,” or “true” value. In other words, individual variation was conceived exclusively as the result of errors introduced in the process of measurement. At its extreme, the mean value would describe an effect that did not apply to any of the individuals studied. In this paradigm, the approach to gaining insight into the mechanisms underlying disease was based on the comparison of mean values between patients and healthy controls, usually resulting from a one-off experiment. Thus, in order to test an involvement of the opioidergic system in depressive disor-

ders, we compared neuroendocrine and behavioral responses to the highly potent μ opiate receptor agonist fentanyl, both in patients and controls. At the time, insights into central receptor functions in humans were to be gained only indirectly: pharmacological substances known to interact with central nervous system receptors were administered intravenously, and receptor-mediated effects such as the release of hormones were measured peripherally as indicators of receptor function.

Evidence for individual variation in response to μ opiate receptor agonist administration

In order to prepare the ground for such an opiate challenge in patients, we had performed, first, a systematic dose-response study in normal volunteers. Doses of 0.1, 0.2, and 0.25 mg fentanyl per 70 kg body weight were tested in a randomized design at 3-week intervals, and specific dose-related effects on the release of prolactin, growth hormone, cortisol, catecholamines, and euphoric responses were able to be demonstrated. In particular, this work presented the first experimental evidence of a dose-dependent increase in the rewarding properties of fentanyl. A dose of 0.2 mg

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per 70 kg body weight proved suitable to reliably induce an opiate-specific effect without causing adverse side effects or stress reactions.¹⁻⁴ When this dose was administered to depressive patients in a one-off experiment, both mean growth hormone and euphoric response to fentanyl was significantly reduced compared with normal controls.⁵ This suggested a possible involvement of μ opioid receptor-related function in depression.

Most interestingly, when the individual responses underlying the mean euphoric effect of fentanyl in normal volunteers (*Figure 1A*) were examined, a remarkable individual variation was observed (*Figure 1B*). One fourth of the “normal” volunteers did not exhibit any euphoric reaction, or showed a decrease in well-being. Evaluation of euphoric responses was based on: (i) application of visual analogue scales; (ii) documentation and classification of all spontaneous verbal reports of the volunteers; and (iii) detailed documentation of all observations during the experiment by two experts. These different instruments were found to be highly concordant, allowing unambiguous classification of the volunteers’ behavioral patterns. Moreover, these individual response patterns proved consistently evocable over time, ie, in the course of repeated applications of fentanyl.^{2,6} This suggested that individual responsiveness to this μ opiate receptor agonist might represent a trait variable, and that “normal” individuals might be classified into drug responders and nonresponders (*Figure 1C*).² Similar observations were made upon administration of morphine.² This suggests that a subgroup of individuals may not be disposed to experience euphoria upon exposure to addictive drugs. Absence of euphoric response was not correlated with a blunted growth hormone release upon application of fentanyl or morphine, suggesting that different (opiate) mechanisms might be involved in mediation of rewarding properties of addictive substances. Thus, tracking a potential genetic basis underlying nonresponse may provide important clues the mechanisms involved in the development of addiction, or in a more general way the personal disposition to experience reward, and potentially lead to targets of intervention.

Evidence for abundant DNA sequence variability in the gene encoding the human μ opiate receptor

Major advances in human molecular genetics in the late 1980s led to the cloning of numerous genes encoding

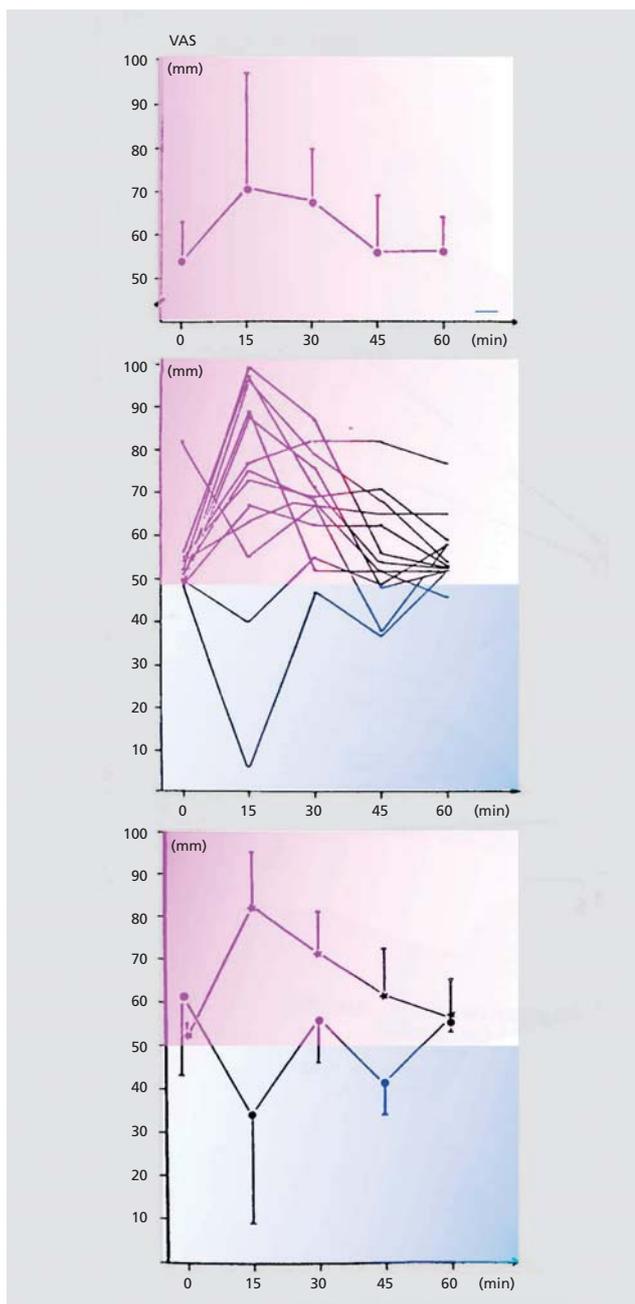


Figure 1. Euphoric responses to μ opiate receptor agonist administration. A) Visual analogue scale (VAS) scores as mean values before and up to 60 min after administration of 0.2 mg fentanyl/kg; 0 mm = very unpleasant feelings; 100 mm = extremely positive feelings B) Visual analogue scale scores presented as individual values before and up to 60 min after administration of 0.2 mg fentanyl/kg C) Classification of individual VAS scores into two response types, euphoric responders / nonresponders.

pharmacologically characterized receptors. This allowed in principle to address the role of receptors in disease and individually different drug response for the first time at the most basic level, that is, DNA sequence information. If DNA sequence differences in the receptor gene were identified that were correlated with the individual phenotype in question, this could provide important clues on underlying receptor dysfunction and its nature. Since it is the entire gene and its encoded protein that act as the units of function which potentially affect a phenotype (and ultimately allow the first conclusions on disease mechanisms), it appeared mandatory to analyze the entire sequences of the individual genes, including their regulatory and critical intronic sequences. This required DNA sequence analyses at a previously unprecedented scale, in the Megabase range. Thus, we developed a powerful technique to perform comparative candidate gene sequencing in large numbers of patients and controls, "Multiplex Polymerase Chain Reaction (PCR) Sequencing." In principle, this technology allowed processing multiple (up to 55) sequencing reactions simultaneously in one reaction tube, increasing throughput accordingly. As a second prerequisite, we generated significant information on the genomic organization of the human μ opiate receptor gene, extending the previously

cloned complimentary DNA (cDNA) sequence information⁷ significantly. We determined several kb of 5' regulatory region, identified a number of potential binding sites for transcriptional regulatory factors, and cloned critical intronic sequences.⁸

These lines of research and technology development were combined to conduct the first systematic and to date most comprehensive analysis of DNA sequence variation in the human μ opiate receptor gene (OPRM1).⁹ In a total of 250 individuals with a phenotype of severe substance (heroin/cocaine dependence and controls from two major populations, African-Americans and European-Americans, abundant DNA sequence diversity was revealed (Figure 2). Regarding the nature and distribution of sequence variation in OPRM1, a total of 43 biallelic variants were identified. Clearly, the density of variants was higher in the 5' regulatory and untranslated regions than in the coding regions, where six variants, five of which affect the encoded protein, were found. Functional analyses of several of these mutations in the coding were performed, characterizing in particular modification of receptor density and signaling (Figure 3).¹⁰ Moreover, the influence of allelic variation in the 5' region on regulation of OPRM1 transcription was analyzed in a first study.¹¹

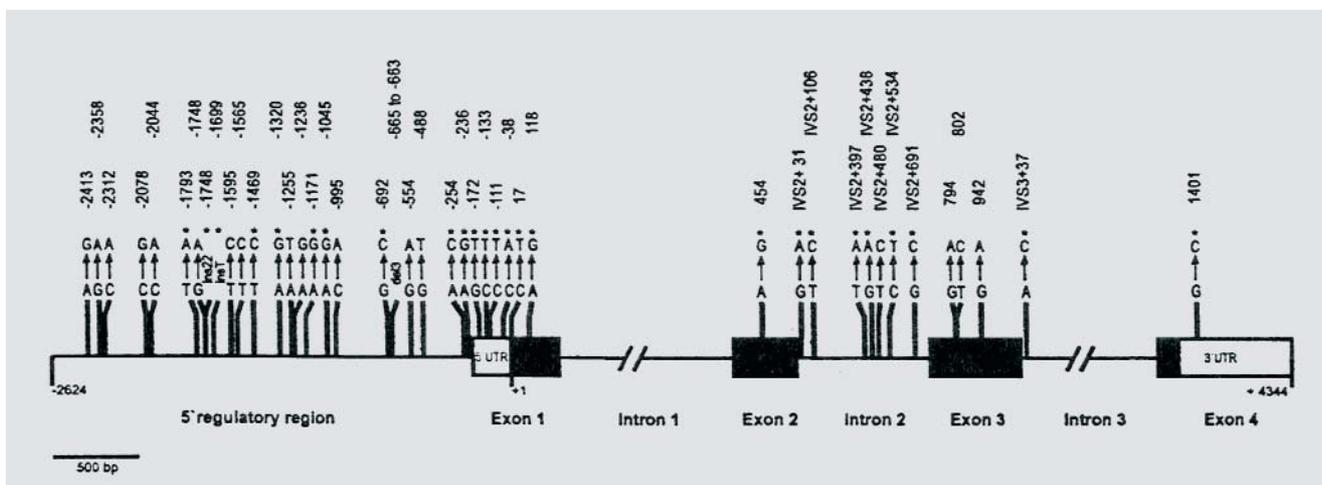


Figure 2. Polymorphic spectrum of the OPRM1 gene. The 6968 bp genomic reference sequence is presented as baseline; base pair coordinates relative to the translation start site are given. Sequences are drawn to scale, which is indicated. All gene variants are specified by position numbers and nucleotide variations (substitutions, insertions and deletions) according to mutation nomenclature. Those sites marked by an asterisk have been included in the haplotype analysis.

Reproduced from ref 9: Hoehe MR, Köpke K, Wendel B, Flachmeier C, Kidd KK, Berrettini WH, Church GM. Sequence variability and candidate gene analysis in complex disease: association of μ opiate receptor gene variation with substance dependence. *Hum Mol Genet.* 2000;19:2895-2908. Copyright © IRL Press at Oxford University Press 2000

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Multiple individually different forms of the human μ opiate receptor gene: relationship to gene function and phenotype

The given sequence variability gives rise to numerous individually different forms of the OPRM1 gene. It is essential in diploid organisms to determine the specific combinations of given gene sequence variants for each of the chromosomes defined as haplotypes. Because current experimental methods to determine the molecular haplotypes are still too labor- and cost-intensive, statistical techniques were applied at this stage to predict these. In the group of African-American substance-dependent individuals and controls, a total of 52 different haplotypes were distinguished (Figure 4A).^{9,12} These occurred at different frequencies in the population, as illustrated in Figure 4B. The five most frequent haplotypes, nos 43, 14, 4, 24, and 7 were common to both substance-dependent individuals and controls and constituted 66% to 73% of all haplotypes. An additional four of less frequent haplotypes were predicted, and a large number (43) of rare haplotypes occurring at frequencies <1% amounted to a total of 20% of all haplotypes. Thus, we will have to abandon Mendel's two-allele concept of a gene, which implicated existence of both a predominant "wild type" and various mutant forms.

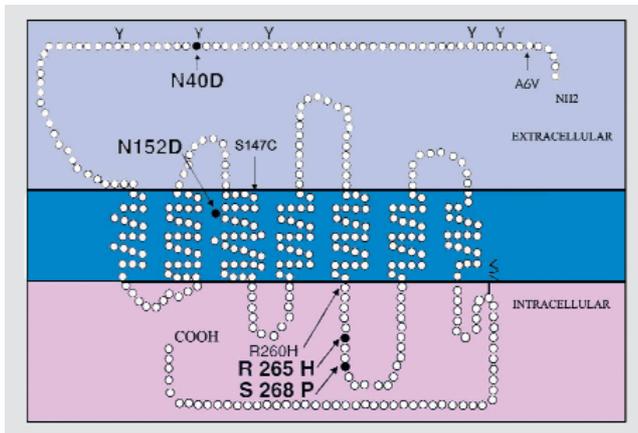


Figure 3. Site-directed mutagenesis of amino acid residues of OPRM1. A schematic representation of the putative seven transmembrane domain topology of the receptor is shown. Polymorphisms that affect protein sequence are indicated, and the mutations examined highlighted.

Reproduced from ref 10: Befort K, Filliol D, Decaillet FM, Gaveriaux-Ruff C, Hoehe MR, Kieffer BL. A single nucleotide polymorphic mutation in the human μ -opioid receptor severely impairs receptor signaling. *J Biol Chem.* 2001;276:3130-3137. Copyright © American Society for Biochemistry and Molecular Biology 2001

The picture exemplified at the model of OPRM1 apparently applies, in view of our more extended candidate gene analyses, to at least one third of all genes studied.¹³ Allelic complexity in candidate genes may be large, and pose particular challenges to the analysis of genotype-phenotype relationships, particularly in the situation of complex traits. At first sight, such multiplicity of gene forms seems irreconcilable with the assumption of dichotomous traits such as health and disease, or drug response and nonresponse. Moreover, the number of different haplotypes is unfeasibly large, so that the power is not sufficient to detect an

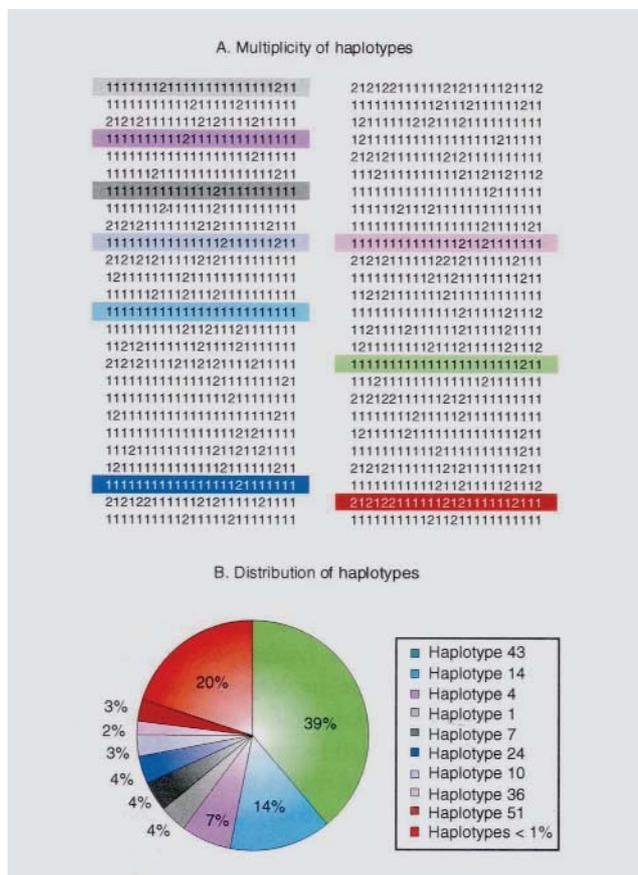


Figure 4. The human μ opiate receptor study. A. The multiplicity of haplotypes. The polymorphic sites are specified by positions 1–25, marked by an asterisk in Figure 2; 1, identical with the reference sequence; 2, different from the reference sequence. B. Distribution of haplotypes. Haplotype frequencies are given in percentages, different haplotypes are color-coded and correspond to the haplotypes marked in A.

Reproduced in part from ref 12: Hoehe MR. Haplotypes and the systematic analysis of genetic variation in genes and genomes. *Pharmacogenomics.* 2003;4:547-570. Copyright © Future Medicine Ltd 2003

association with any single haplotype. This will require novel approaches to cope with the multiplicity of haplotypes. An appropriate approach seems the classification of haplotypes into functionally related (ideally functionally equivalent) ones based on sequence-structure-function similarity. Once a classification has been derived, the haplotype frequencies of cases and controls in the different classes can be compared. By this approach, the multiplicity of haplotypes could be condensed to two functionally related categories, one of which was more frequent in substance-dependent individuals.⁹ Common to this category was a characteristic pattern of sequence variants located in the 5' regulatory region, reflecting a specific constellation of putative transcription regulatory motifs that may confer different regulatory properties.^{9,12} Taken together, this analysis at the gene level demonstrates a remarkable gene sequence and haplotype diversity, the rule rather than the exception for the majority of candidate genes. This work provides, moreover, an example of approaches that can be successfully applied to establish complex genotype-phenotype relationships against a background of high natural genome sequence diversity.

Perspectives

Observed diversity presents challenges to the traditional views of the concept of "a" gene with far-reaching implications on the analysis of "gene"- "function" relationships.^{13,14} Classical single mutation analysis no longer appears appropriate. The units of functional analysis must

be the entire individual sequence of haplotypes, involving potentially abundant variation in *all* regulatory, coding, and intronic sequences. Analysis will include the spectrum of haplotypes existing in a population, and the pairs of haplotypes existing in each individual. We have now determined in a first comprehensive study the molecular haplotypes of a key candidate gene in hundreds of individuals, confirming the existence of multiple individually different forms of a gene at the molecular level (Hoehe et al, in preparation). This work provides at the same time knowledge of the concrete molecular templates to allow dissection of what may be an entire spectrum of functions underlying molecular gene diversity.

At this stage, individual variation and its functional implications have been addressed at the level of a single gene only. However, this is integral part of an entire network of genes as a higher-level functional unit; multiple individual molecular haplotypes interact to produce a common output signal. Thus, progress in the future is expected to come from whole systems analysis-based approaches,¹³ integrating individual variation in all genes involved in all pathways of relevance. This will prepare the basis for "personalized" medicine in its true sense. □

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REFERENCES

1. Hoehe M, Duka T, Doenicke A, Matussek N. Dose-dependent influence of fentanyl on prolactin, growth hormone, and mood. *Neuropeptides*. 1984;5:269-272.
2. Hoehe M. *Effects of Opiates on Neuroendocrine and Behavioural Parameters*. [doctoral thesis] Munich, Germany: Ludwig-Maximilians-University; 1984.
3. Hoehe M, Duka T, Doenicke A. Human studies on the mu opiate receptor agonist fentanyl: neuroendocrine and behavioral responses. *Psychoneuroendocrinology*. 1988;13:397-408.
4. Hoehe M, Duka T. Opiates increase plasma catecholamines in humans. *Psychoneuroendocrinology*. 1993;18:141-148.
5. Matussek N, Hoehe M. Investigations with the specific mu opiate receptor agonist fentanyl in depressive patients: growth hormone, prolactin, noradrenaline, cortisol and euphoric responses. *Neuropsychobiology*. 1989;21:1-8.
6. Hoehe M. Influence of the menstrual cycle on neuroendocrine and behavioral responses to an opiate agonist in humans. Preliminary results. *Psychoneuroendocrinology*. 1988;13:339-344.
7. Wang JB, Johnson PS, Persico AM, Hawkins AL, Griffin CA, Uhl GR. Human mu opiate receptor. cDNA and genomic clones, pharmacologic characterization and chromosomal assignment. *FEBS Lett*. 1994;338:217-22.
8. Wendel BC, Hoehe MR. The human μ opioid receptor gene: 5' regulatory and intronic sequences. *J Mol Med*. 1998;76:525-532.
9. Hoehe MR, Köpke K, Wendel B, et al. Sequence variability and candidate gene analysis in complex disease: Association of μ opioid receptor gene variation with substance dependence. *Hum Mol Genet*. 2000;19:2895-2908.
10. Befort K, Filliol D, Decaillet FM, Gaveriaux-Ruff C, Hoehe MR, Kieffer BL. A single nucleotide polymorphic mutation in the human μ -opioid receptor severely impairs receptor signaling. *J Biol Chem*. 2001;276:3130-3137.
11. Kraus J, Börner C, Giannini E, et al. Regulation of mu opioid receptor gene transcription by interleukin-4 and influence of an allelic variation within a STAT6 transcription factor binding site. *J Biol Chem*. 2001;276:43901-43908.
12. Hoehe MR. Haplotypes and the systematic analysis of genetic variation in genes and genomes. *Pharmacogenomics*. 2003;4:547-570.
13. Hoehe MR, Timmermann B, Lehrach H. Human inter-individual DNA sequence variation in candidate genes, drug targets, the importance of haplotypes and implications for pharmacogenomics. *Curr Pharm Biotechnol*. 2003;4:351-378.
14. Hoehe MR, Krosiak T. Genetic variation and pharmacogenomics: concepts, facts, and challenges. *Dialogues Clin Neurosci*. 2004;6:5-25.