ELSEVIER

European Journal of Pharmacology 410 (2000) 249–268

ejp

www.elsevier.nl/locate/ejphar

Opioid receptor and peptide gene polymorphisms: potential implications for addictions

K. Steven LaForge *, Vadim Yuferov, Mary Jeanne Kreek

Laboratory Biology of Addictive Diseases, The Rockefeller University, Box 171, 1230 York Avenue, New York, NY 10021, USA

Accepted 20 October 2000

Abstract

Addictions to drugs of abuse and alcohol have been shown by studies of genetic epidemiology to have both a heritable and an environmental basis, with these factors influencing addiction to different substances to a different extent. In the search for specific alleles of specific genes that may contribute to the development of the addictions, many researchers have focused on the endogenous opioid system, which mediates a diverse array of neurological, physiological, and behavioral functions. The endogenous opioid system is also centrally important in mediating the effects of drugs of abuse and alcohol. Polymorphisms, including single nucleotide polymorphisms, have been identified in genes of the endogenous opioid receptors and peptides. A number of recent genetic association studies and a few studies of potential function provide clues as to which genes and which alleles may have implications for human physiology and pathophysiology, including the addictions.

Keywords: Opioid system, endogenous; Gene polymorphism; Single nucleotide polymorphism; Addiction; Pharmacogenetics; Physiogenetics

1. Introduction

1.1. Epidemiological studies of the genetics of addictions

Many individuals are self-exposed to alcohol and drugs of abuse, and many continue to use alcohol or illicit drugs on an occasional, or even regular, basis; however, only some individuals develop specific addictions. For example, the latest report from the 1999 National Household Survey on Drug Abuse indicate that over 3 million persons in the United States have used heroin at some time in their life (SAMHSA, 2000); current estimates of heroin addicts in that country range from 0.8 to 1.2 million. Why do some become addicted following self-exposure while others do not?

Differences in individual variability to develop specific addictions have both an environmental and a heritable basis. Early twin and adoption studies established a role for heritability in alcoholism (e.g. Kaij, 1960; Partanen et al., 1966; Goodwin et al., 1973; Cloninger et al., 1981; Hrubec and Omenn, 1981; Cadoret et al., 1985). Heterogeneity of inheritance patterns in alcohol abuse disorders have been defined, with the highest heritability estimates for males (Cloninger et al., 1981; Pickens et al., 1991). More recently, family, twin, and adoption studies have demonstrated that other substance abuse disorders also have, at least in part, a genetic basis (e.g. Cadoret et al., 1986; Grove et al., 1990; Tsuang et al., 1996; Bierut et al., 1998; Merikangas et al., 1998; Kendler and Prescott, 1998; Tsuang et al., 1998; Kendler et al., 1999). In a recent study of 3372 twin pairs, for example, evidence was found for a shared or common vulnerability for abuse of several types of drugs, with influences of genetic, family environmental, and non-family environmental factors. This shared vulnerability influenced susceptibility to abuse for different drugs to a different extent. In addition to the shared vulnerability, each drug had heritable influences specific to itself. Heroin abuse or dependence was found to have the highest genetic variance (54%), and also the highest degree of unique genetic influence, with 38% of the genetic variance specific to that drug (Tsuang et al., 1998).

1.2. The endogenous opioid system and vulnerability to specific addictions

Although a heritable basis for addictions has been established, the specific genes involved in the etiology of these

* Corresponding author. Tel.: +1-212-327-8234; fax: +1-212-327-8574.

E-mail address: laforgk@rockvax.rockefeller.edu (K.S. LaForge).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00819-0

Table 1
Chromosomal localizations of human opioid system genes

Gene	Location
μ-Opioid receptor	6q24–25[a]
κ-Opioid receptor	8q11.2[b,c]
δ-Opioid receptor	1p34.3–36.1[d]
Proopioimelanocortin (POMC)	2p23.3[e,f,g]
Enkephalin	8q23–q24[h]
Dynorphin	20p12-pter[h]

[a] Wang et al. (1994).
[b] Yasuda et al. (1994).
[c] Simonin et al. (1995).
[d] Befort et al. (1994).
[e] Owerbach et al. (1981).
[f] Zabel et al. (1983).
[g] Satoh and Mori (1997).
[h] Litt et al. (1988).

disorders has not been well defined. Researchers have hypothesized that specific combinations of alleles of specific genes may result in innate differences in phenotypic expression of cellular or physiological systems known to be important in mediating the responses of drugs of abuse and alcohol; other studies have shown that opiates, cocaine, other drugs of abuse and alcohol profoundly alter physiological and cellular systems (see Kreek, 1996a,b; Kreek and Koob, 1998; Kreek, 2000). These changes are specific for the route and pattern of administration and length of time of exposure. Some of the induced alterations may be long lasting or even permanent. Therefore, cellular or physiological systems which show alterations in response to drugs of abuse and alcohol, and which might respond differently in individuals due to innate genetic differences that result from polymorphisms of any type, may also underlie the development of these disorders. One such system, the endogenous opioid system, is central in mediating the neurobiological and physiological responses to drugs of abuse and alcohol. Individual genetic differences that lead to functional differences in the endogenous opioid system may therefore be of interest as subjects for genetic studies of vulnerability to specific addictions. Human chromosomal locations for the identified endogenous opioid system genes (for the mu (μ-), delta (δ-), and kappa (κ-) opioid receptors and proopioimelanocortin (POMC), proenkephalin, and prodynorphin), are shown in Table 1. (For recent reviews of the endogenous opioid system and its functions, see Kreek, 1996b; Nestler and Aghajanian, 1997; Standifer and Pasternak, 1997; Akil et al., 1997; Massotte and Kieffer, 1998; Shippenberg and Elmer, 1998; Connor and Christie, 1999; Law and Loh, 1999.)

2. Single nucleotide polymorphisms and other polymorphisms in human opioid system genes

2.1. Polymorphisms in the human μ-opioid receptor gene

Because of the physiological significance of the μ-opioid receptor, particularly with respect to its role in mediating the analgesic and rewarding effects of opiate drugs, and also because of the evidence from quantitative trait locus (QTL) and knockout studies in mice (e.g. Belknap and Crabbe, 1992; Belknap et al., 1995; Berrettini et al., 1994; Matthes et al., 1996; Roberts et al., 2000), investigators have focused attention on this gene as a candidate in studies of polymorphism and potential association of allelic variants with opiate and other addictions, including alcoholism. Several recent reports have identified polymorphisms in this gene, including single nucleotide polymorphisms (frequently now abbreviated "SNPs"). Single nucleotide polymorphisms of this gene are summarized in Table 2; those that are found at positions of coding region of the genes are identified in Fig. 1. Numerous diverse numbering systems for nucleotides and amino acids have been used in the published reports included in this review. While some are based on functional considerations, others are based on the arbitrary numbering of a specific sequence in an archival report. In an effort to achieve consistency, the numbering of nucleotides and amino acids for the polymorphisms reported on herein has been modified according to the following system: positions for single nucleotide polymorphisms in coding regions, 5′ and 3′ untranslated regions and flanking sequences are based on assignment of +1 to the first nucleotide in the ATG initiation codon of the receptor or prepropeptide; numbering of single nucleotide polymorphisms within a specific intron (IVS or intervening sequence) is based on assignment of +1 to the first noncoding nucleotide of that intron; for each single nucleotide polymorphism, the nucleotide substitution that is identified as the most common or accepted prototype is given before the nucleotide position. For all receptors and peptide precursors, the initiation methionine of the prepropeptide is assigned amino acid number +1.[1]

In the first published report, Berrettini et al. (1997) used single-stranded conformational polymorphism analysis and confirmatory DNA sequencing and identified two common single nucleotide polymorphisms in exon I of the μ-opioid receptor gene in 55 cocaine and/or opioid dependent individuals and 51 matched control subjects. The two polymorphisms identified were G-38T in the 5′ untranslated region and C17T in the coding region. The C17T polymorphism results in a predicted amino acid substitution (Ala6Val) in the N-terminal domain of the receptor. The variant-38T and 17T alleles had overall allele frequencies of 0.061 (6.1%) and 0.160 (16.0%), respectively, in the populations studied. The authors reported that they

[1] This renumbering of previously published data led to the identification of inconsistencies in some reports. In most cases, information presented was sufficient to resolve these. If inconsistent data could not resolve the position of a polymorphism, it has not been included in this report.

Table 2
Single nucleotide polymorphisms in the human μ-opioid receptor gene

Nucleotide substitution[a]	Location in gene	Amino acid substitution[b]	Functional domain	Detection method[c]	Overall allelic frequency reported
G-54T	Exon I	NA	5′ untranslated region	A	Not reported[d]
G-38T	Exon I	NA	5′ untranslated region	A, B	0.061[e]
C12G	Exon I	Ser4Arg (S4R)	N-terminal	A	Not reported[d]
C17T	Exon I	Ala6Val (A6V)	N-terminal	A, B	0.160[e]
				A	< 0.01[f]
				A	0.066[g]
				C	0.036[h]
G24A	Exon I	Synonymous (Thr8)	N-terminal	A	0.020[g]
A118G	Exon I	Asn40Asp (N40D)	N-terminal	A, C	0.139[f]
				A	0.105[g]
				D	0.092[i]
				A	Not reported[d]
				C	0.130[j]
				C	0.141[h]
				D	0.093[k]
				C	0.321[l]
C440G	Exon II	Ser147Cys (S147C)	Transmembrane domain 3	A	< 0.01[f]
				A	Not reported[d]
A454G[m]	Exon II	Asn152Asp (N152D)	Transmembrane domain 3	A	Not reported[n]
IVS2 G31A	Intron 2	NA	NA	A	Not reported[o]
IVS2 C691G	Intron 2	NA	NA	A, C	0.425[f]
				C	0.265[l]
G779A	Exon III	Arg260His (R260H)	Cytoplasmic loop 3	A	< 0.01[g]
G794A[m]	Exon III	Arg265His (R265H)	Cytoplasmic loop 3	A	Not reported[n]
T802C[m]	Exon III	Ser268Pro (S268P)	Cytoplasmic loop 3	A	Not reported[n]
G942A	Exon III	Synonymous (Thr314)	Extracellular loop 3	A	< 0.01[g]

Abbreviations: NA, not applicable.

[a] Numbering system for single nucleotide polymorphisms in the coding region and 5′ or 3′ untranslated regions is based on assignment of +1 to the first nucleotide in the ATG initiation codon of the receptor. Numbering of single nucleotide polymorphisms in intron sequences (IVS or intervening sequence) based on assignment of +1 to the first noncoding nucleotide of that intron. Nucleotide substitution identified as the most common or from an accepted prototype sequence is given before the substituted nucleotide position. Prototype sequence used in numbering: GenBank accession number L29301.

[b] The initiation methionine of the prepropeptide is assigned amino acid number +1.

[c] Detection methods used in the cited report: (A) sequencing of PCR amplified DNA, (B) single strand conformation polymorphism analysis, (C) PCR-restriction fragment polymorphism analysis, and (D) allele-specific PCR amplification.

[d] Uhl et al. (1999).

[e] Berrettini et al. (1997).

[f] Bergen et al. (1997).

[g] Bond et al. (1998).

[h] Gelernter et al. (1999).

[i] Sander et al. (1999).

[j] Town et al. (1999).

[k] Smolka et al. (1999).

[l] Li et al. (2000).

[m] Nucleotide substitution not reported. Position and base substituted was deduced from reported amino acid substitution.

[n] Befort et al. in LaForge and Kreek (2000).

[o] Wendel and Hoehe (1998).

screened all four exons of the μ-opioid receptor gene using single strand conformation polymorphism analysis and DNA sequencing and did not identify any other allelic variants with a minor allelic frequency of greater than 0.05.

In a study of 398 alcohol dependent individuals and 393 control subjects, Bergen et al. (1997) used DNA sequencing of polymerase chain reaction (PCR) amplified genomic DNA to examine 91% (1093 bases) of the coding sequence and an additional 1479 bases of intron and untranslated sequences in 30 chromosomes, with exon I sequences determined in an additional 104 chromosomes. In this initial screen, four single nucleotide polymorphisms were identified, including the previously reported C17T substitution, as well as the variants A118G, C440G, and IVS2 C691G. The C17T and C440G polymorphisms were identified as "rare" with allelic frequencies below 0.001. In contrast, using DNA sequencing and PCR amplification of genomic fragments followed by restriction fragment length polymorphism assays, the A118G and IVS2 C691G poly-

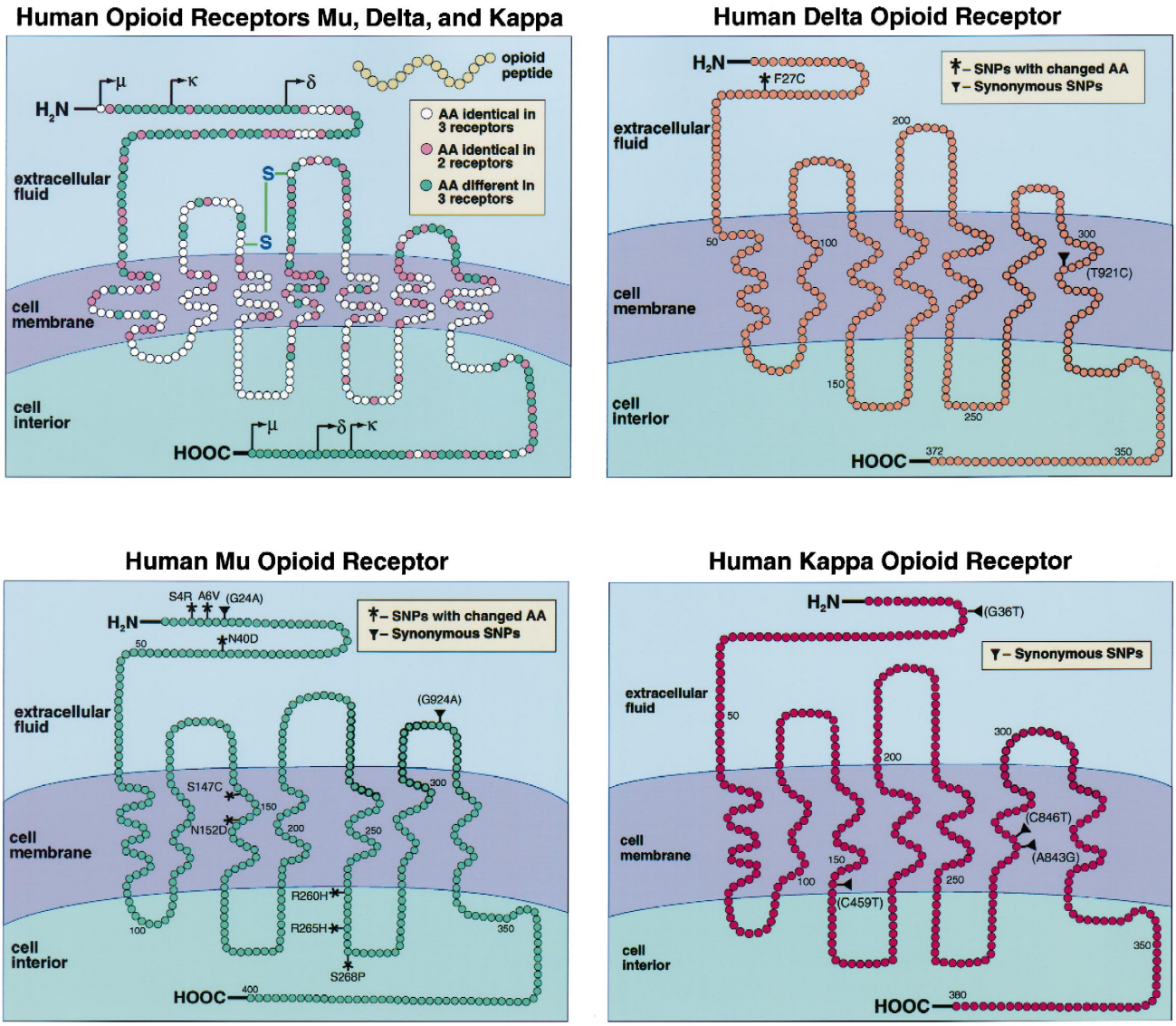

Fig. 1. The human mu (μ-), delta (δ-) and kappa (κ-) opioid receptors and positions of identified coding region single nucleotide polymorphisms. Predicted positions of the extracellular domains (N-terminal, extracellular loops one through three), seven transmembrane domains, and intracellular domains (carboxy-terminal, intracellular loops one through three) are indicated. The upper right panel shows a composite drawing of the three opioid receptors, (μ-, δ-, and κ-), showing the extent of amino acid homology, and differences in N-terminal and carboxy-terminal length for each receptor type. The position of a disulfide bond between the first and second extracellular loops is indicated in the composite drawing. Diagram was prepared using comparisons of amino acid sequences reported in Knapp et al. (1995). Individual receptors are shown in the three additional panels, with positions of coding region single nucleotide polymorphisms indicated. (For literature citations of currently identified single nucleotide polymorphisms, see Tables 2 and 3.) Abbreviations: SNP, single nucleotide polymorphism.

morphisms had overall allelic frequencies of 0.139 and 0.425, respectively. The A118G polymorphism had not been previously identified as a polymorphic variant in published reports, however, the possibility of a guanine substitution at position 118 could be identified by a sequence discrepancy among reported GenBank entries L25119, U12569, and L29301, for the human μ-opioid receptor gene or cDNA sequence (Wang et al., 1994; Bare et al., 1994; Mestek et al., 1995). Like the C17T substitution, the A118G and C440G variants result in amino acid substitutions in the predicted primary structure of the receptor (Asn40Asp and Ser147Cys).

In a study of 113 opioid dependent individuals and 39 control subjects, we identified five single nucleotide polymorphisms in the coding region of the μ-opioid receptor gene by sequencing PCR amplified DNA (Bond et al., 1998). Identified polymorphisms included the C17T and A118G substitutions, both of which were found to be common in the study populations, with overall allelic frequencies of 0.066 and 0.105 for the 17T and 118G alleles, respectively. One additional single nucleotide polymorphism was identified in exon I, the synonymous G24A substitution, with an overall allelic frequency of 0.020. Two rare single nucleotide polymorphisms with allele

frequencies below 0.01 were identified in exon III: the G779A substitution, which leads to a predicted Arg260His substitution in the third intracellular domain of the receptor and the synonymous G924A substitution. The A118G polymorphism was identified as being of particular significance in this report, since the variant receptors encoded by different alleles have potential differences in cellular functioning (see below).

The apparent differences in identified allele frequencies for the C17T and A118G single nucleotide polymorphisms between these three reports might be due either to differences in the methods used or to actual differences in allele frequencies among the populations studied. In particular, Berrettini et al. (1997), using single strand conformation polymorphism detection, did not identify the A118G polymorphism in any of the subjects they studied, although this region of the gene was examined. This finding may have resulted from a failure of the technique used. Methods of identification of polymorphisms that rely on conformational differences in electrophoretic mobility, such as single strand conformation polymorphism analysis, fail to identify 10% to 20% of SNPs, and do so systematically if identical experimental conditions are used (e.g. Jordanova et al., 1997; Cargill et al., 1999).

Additional studies using PCR-restriction fragment length polymorphism assays have reported on population distribution or disease associations of the C17T and A118G polymorphisms; these are listed in Table 2 (Gelernter et al., 1999; Smolka et al., 1999; Town et al., 1999; Li et al., 2000).

Several additional single nucleotide polymorphisms have been identified in the μ-opioid receptor gene, including IVS2 G31C (Wendel and Hoehe, 1998); and G-54T and C12G (Uhl et al., 1999). Allelic frequencies for these polymorphisms were not reported. In a very recent study of a cohort of 450 individuals, we identified four additional novel single nucleotide polymorphisms in the first exon of the μ-opioid receptor gene (unpublished observations).

Other types of polymorphic variants of the human μ-opioid receptor gene have also been identified. In their initial paper describing the cloning of human μ-opioid receptor cDNA and a gene fragment, Wang et al. (1994) describe an *Msp* I restriction fragment length in 31 of 49 subjects studied. A less common *Msp* I restriction fragment length polymorphism variant found in 2 of 49 subjects is also described in this report. Using DNA sequencing, Uhl et al. (1999) identified several other variants of the gene including a dinucleotide polymorphism in the coding region (CG912GC), and, in noncoding sequences, five dinucleotide repeats, a trinucleotide repeat, and a short simple repeat. Allele frequencies of these polymorphisms are not stated in this report. A variable dinucleotide repeat (occurring in nine allelic forms, from 7 to 21 copies) in an undisclosed noncoding position in the gene has also been investigated in both population and addictive disease studies (Gelernter et al., 1998; Kranzler et al., 1998). In very recent studies, we have found a three base deletion in exon I coding region sequence in several individuals.

2.2. Polymorphisms of the human δ-opioid receptor gene

Two single nucleotide polymorphisms of the human δ-opioid receptor genes have also been recently identified (Mayer et al., 1997; Franke et al., 1999; Höllt in LaForge and Kreek, 2000). The positions of these are summarized in Table 3 and indicated in Fig. 1. These sequence variants have been verified as single nucleotide polymorphisms, since they are found in specific study subjects; their exis-

Table 3
Single nucleotide polymorphisms in the human δ- and κ-opioid receptor genes

Nucleotide substitution[a]	Location in gene	Amino acid substitution[a]	Functional domain	Detection method[b]	Overall allelic frequency reported
(A) Human δ-opioid receptor gene					
T80G[c]	Exon I	Phe27Cys (F27C)	N-terminal	A	0.10[d]
T921C[c]	Exon III	Synonymous (Gly307)	Transmembrane domain 7	A, C	0.459[e]
				C	0.368[f]
(B) Human κ-opioid receptor gene					
G36T	Exon I[g]	Synonymous (Pro12)	N-terminal	A	< 0.05[d]
C459T	Exon II	Synonymous (Ser153)	Transmembrane domain 3	A	< 0.05[d]
A843G	Exon III	Synonymous (Ala281)	Transmembrane domain 6	A	< 0.05[d]
C846T	Exon III	Synonymous (Val282)	Transmembrane domain 6	A	< 0.05[f]

[a] Numbering system for single nucleotide polymorphisms and amino acids is as defined in Table 2. Prototype sequences used in numbering: δ-, GenBank accession number U10504; κ-, GenBank accession number U17298.

[b] Detection methods used in the cited report: (A) sequencing of PCR amplified DNA, (C) PCR-restriction fragment polymorphism analysis.

[c] Nucleotide substitution not reported. Position and base substituted was deduced from reported amino acid substitution.

[d] Höllt in, LaForge and Kreek (2000).

[e] Mayer et al. (1997).

[f] Franke et al. (1999).

[g] Numbering of exons is as described in Simonin et al. (1995).

tence could also be predicted from sequence discrepancies at these positions identified in GenBank entries U07882, U10504, and AL009181 for the human δ-opioid receptor.

2.3. Polymorphisms in the human κ-opioid receptor gene

Four single nucleotide polymorphisms in the human κ-opioid receptor gene have been recently identified and are listed in Table 3, with positions also indicated in Fig. 1 (Höllt in LaForge and Kreek, 2000). These polymorphisms have been demonstrated to exist in specific individuals, although possible polymorphisms at these positions could also be predicted from sequence discrepancies recorded in GenBank entries U11053 and U17298 for the κ-opioid receptor. Very recently, we have also verified that the previously identified G36T, A843G, and C846T substitutions are polymorphisms found in individuals and also identified three previously undefined single nucleotide polymorphisms in the coding region of the gene (unpublished observations).

2.4. Polymorphisms in the human POMC gene

Using a probe that contained the first exon of the POMC gene, Feder et al. (1983) screened DNA from 20 unrelated individuals and identified a high frequency, two allele, *Sst* I RFLP within 10 kb of the POMC gene. DNA from 146 individuals from 28 families was screened for the polymorphism, which had an observed frequency of 0.278. In a second report, Feder et al. (1985) analyzed DNA for RFLPs at the *POMC* locus in 33 patients with schizophrenia, 22 patients with bipolar disorder, and 67 normal control subjects. In addition to the *Sst* I RFLP, the authors reported an *Rsa* I RFLP at this locus with an allele frequency of 0.34 for the minor allele in 45 control individuals tested.

Gostout et al. (1993), using PCR and DNA sequencing, studied a cryptic trinucleotide repeat of the form purine, purine, pyrimidine (cRRY(i)) in exon III of the POMC gene from nucleotide position 271 to 297 of the prototype sequence reported by Takahashi et al. (1981, 1983). Three polymorphic alleles of this 27 base repeat were described in a sequencing screen of six individuals. The allelic variation did not alter the predicted amino acid sequence of the propeptide (AsnSerSer SerSerGly SerSerGly or NSS SSG SSG). Morris et al. (1993) sequenced a pituitary cDNA POMC clone and identified a nine base deletion at the site of this cryptic repeat, which is in the region of the gene that encodes the 16K peptide, carboxy-terminal to the γ-melanocyte stimulating hormone (γ-MSH) peptide sequences. In a subsequent study of 90 unrelated individuals, this group went on to define a third allele resulting from an 18 base deletion at this site (Morris et al., 1994). Allele frequencies of the three alleles (designated A1, A2, and A3) were 0.02, 0.73, and 0.25 for the 27 base (prototype), nine base deletion, and 18 base deletion alleles, respectively. It should be noted that the first cloned prototype sequence (Takahashi et al., 1981, 1983) was found to be the rarer of the three allelic forms identified. Also, since these variants alter predicted amino acid structure of the 16K fragment of the propeptide they might be expected to alter function, although they are carboxy-terminal to the mature γ-MSH peptide.

Three additional polymorphic variants of the POMC gene were identified in a recent study of families of individuals with an unusual condition complex predicted to result from deficiencies in POMC derived peptides (early onset obesity, red hair pigmentation, and adrenal insufficiency). single nucleotide substitutions and a single nucleotide deletion of the POMC gene are listed in Table 4 and shown in Fig. 2. In homozygous or compound heterozygous condition, these polymorphisms result in disruption of synthesis of adrenocorticotropic hormone (ACTH), α-melanocyte stimulating hormone (α-MSH) and β-endorphin (Krude et al., 1998). One variant, the G313T single nucleotide polymorphism, results in a premature termination at codon 105 (Glu), which is in the propeptide sequence between γ-MSH and α-MSH. The second polymorphism is a single nucleotide deletion (C433Δ) at codon 145 (Arg), which results in a frame shift which is predicted to disrupt the receptor binding core of ACTH and α-MSH, and also introduces a premature termination codon at amino acid position 157, which precedes the sequence of β-lipotropin (β-LPH). A third polymorphism, the C-11A variant, introduces a frame-shifted translation initiation codon in a favorable translation consensus sequence within a few bases of the correct initiation codon, which in turn disrupts translation of POMC gene products. In this study, allele frequencies of the G313T or C433Δ polymorphisms were not reported; the C-11A substitution was not found in a screen of 50 healthy control subjects, suggesting that it was not a common polymorphism (Krude et al., 1998).

Since POMC derived peptides (particularly α-MSH) are thought to be important in modulation of body weight, several recent association studies of alleles of the POMC gene and obesity have been conducted. In a study of 96 obese children and adolescents, 60 healthy underweight students, and 46 individuals with anorexia nervosa, Hinney et al. (1998) used single strand conformation polymorphism analysis and DNA sequencing of exons II and III and identified several novel polymorphisms including seven novel low frequency single nucleotide polymorphisms (Table 4). Two of the identified variants (G316A and A641G) lead to amino acid changes in the predicted propeptide sequence (Asp106Asn and Glu214Gly, respectively), and one variant (G616T) encodes a premature stop codon at amino acid position 206 (Glu), which is within the sequence of γ-LPH. In one study subject, the authors also identified a novel out-of-frame six base insertion (CCCGGG) which results in the addition of two amino acids (Arg–Ala) within codon 150 (Gln), which is in the γ-LPH peptide, and would also disrupt translation of the remain-

Table 4
Single nucleotide substitutions and single nucleotide deletions in the human POMC gene

Nucleotide substitution[a]	Location in gene	Amino acid substitution[a]	Functional domain	Detection method[b]	Overall allelic frequency reported
G-736C	5′ flanking	NA	5′ flanking sequence	A, B, E	0.019[c]
G-117A	5′ flanking	NA	5′ flanking sequence	A, B, E	0.012[c]
C-11A	Exon II	NA	5′ untranslated region	A, C	< 0.01[d]
C18T	Exon II	Synonymous (Cys6)	Signal peptide	A, B	< 0.01[e]
				A, B, E	0.012[c]
C282T	Exon III	Synonymous (Ser94)	16K fragment[f]	A, B	0.01[e]
G313T	Exon III	Glu105 Stop	16K fragment[f]	A, B	Not reported[d]
G316A	Exon III	Asp106Asn	16K fragment[f]	A, B	0.01[e]
C346T	Exon III	Synonymous (Leu116)	16K fragment[f]	A, B	0.012[c]
C411G	Exon III	Synonymous (Arg137)	16K fragment–ACTH junction	A, B	0.01[e]
C433Δ	Exon III	Arg145 frame shift	ACTH and α-MSH	A, B	Not reported[d]
C585T	Exon III	Synonymous (Ala195)	γ-LPH	A, B	< 0.01[e]
				A, B	Not reported[g]
G616T	Exon III	Glu206 Stop	γ-LPH	A, B	< 0.01[e]
A641G	Exon III	Glu214Gly	γ-LPH	A, B	< 0.01[e]
C866T	Exon III	NA	3′ untranslated region	A, B	0.11[g]
				A, B, C, E	0.183[c]

Abbreviations: NA, not applicable; Stop, termination codon; ACTH, adrenocorticotropic hormone; MSH, melanocyte stimulating hormone; LPH, lipotropin.

[a] Numbering system for single nucleotide polymorphisms and amino acids is as defined in Table 2. Prototype sequence used in numbering: GenBank accession number V01510.

[b] Detection methods used in the cited report: (A) sequencing of PCR amplified DNA, (B) single strand conformation polymorphism analysis, (C) PCR-restriction fragment polymorphism analysis, and (E) heteroduplex analysis.

[c] Echwald et al. (1999).

[d] Krude et al. (1998).

[e] Hinney et al. (1998).

[f] Position of polymorphism is carboxy-terminal to γ-MSH peptide derived from processing of the 16K fragment.

[g] Hixson et al. (1999).

der of the propeptide (including β-endorphin). Also identified in this report are insertions of nine bases and of 18 bases, with overall allelic frequencies of 0.045 and < 0.01, respectively. These insertions are at the position of the previously identified cryptic trinucleotide repeat and result in the SerSerGly (SSG) and SerSerGly SerSerGly (SSG SSG) motifs previously described (Gostout et al., 1993; Morris et al., 1993, 1994). Although the earlier studies of Morris et al. and Gostout et al. identifying this cryptic repeat motif were not cited in this report, the 9 and 18 base insertions described by Hinney et al. (1998) are probably the previously described A1 and A2 alleles of this polymorphism, particularly since the sequence of the repeats (AGC AGC GGC and AGC AGC GGC AGC AGC GGC) are identical to the previously reported sequences (Takahashi et al., 1981, 1983; Gostout et al., 1993; Morris et al., 1994).

Two additional studies on POMC polymorphisms and obesity have recently been reported. Hixson et al. (1999), using restriction fragment length polymorphism, PCR and DNA sequencing, studied potential associations with POMC alleles in 337 individuals from 10 families. In this population, the cryptic trinucleotide repeat polymorphism was not found to be common enough to be useful as a genetic marker. A novel common single nucleotide polymorphism (C866T) located in the 3′ untranslated region was identified, with an overall frequency of 0.11 for the 866T allele. The authors also studied the previously identified *Rsa* I restriction fragment length polymorphism, which they observed at an overall allelic frequency of 0.16. Echwald et al. (1999) identified additional novel POMC single nucleotide polymorphisms in a study of 150 men with juvenile onset obesity, 205 matched controls, and a population sample of 380 individuals (see Table 4 and Fig. 2). In this study, single strand conformation polymorphism and heteroduplex assays were used to screen promoter sequences and the entire coding sequence, including intron/exon boundaries, in a randomly selected group of 56 obese subjects. A PCR assay was also used to determine frequencies of previously described alleles of the cryptic trinucleotide repeat in exon III; overall allele frequencies in 1482 chromosomes assessed were 0.003, 0.071, and 0.926 for the A1, A2, and A3 alleles, respectively (Echwald et al., 1999).

2.5. Polymorphisms in the human enkephalin gene

Several polymorphisms have been identified in the human preproenkephalin gene. A (CA)n polymorphic repeat is located in the 3′ flanking sequence approximately 300 bases downstream from the polyadenylation signal (Weber and May, 1990). This study reported five alleles with frequencies varying from 0.01 to 0.54 in 53 Caucasian individuals. Two other studies examined addictive disease

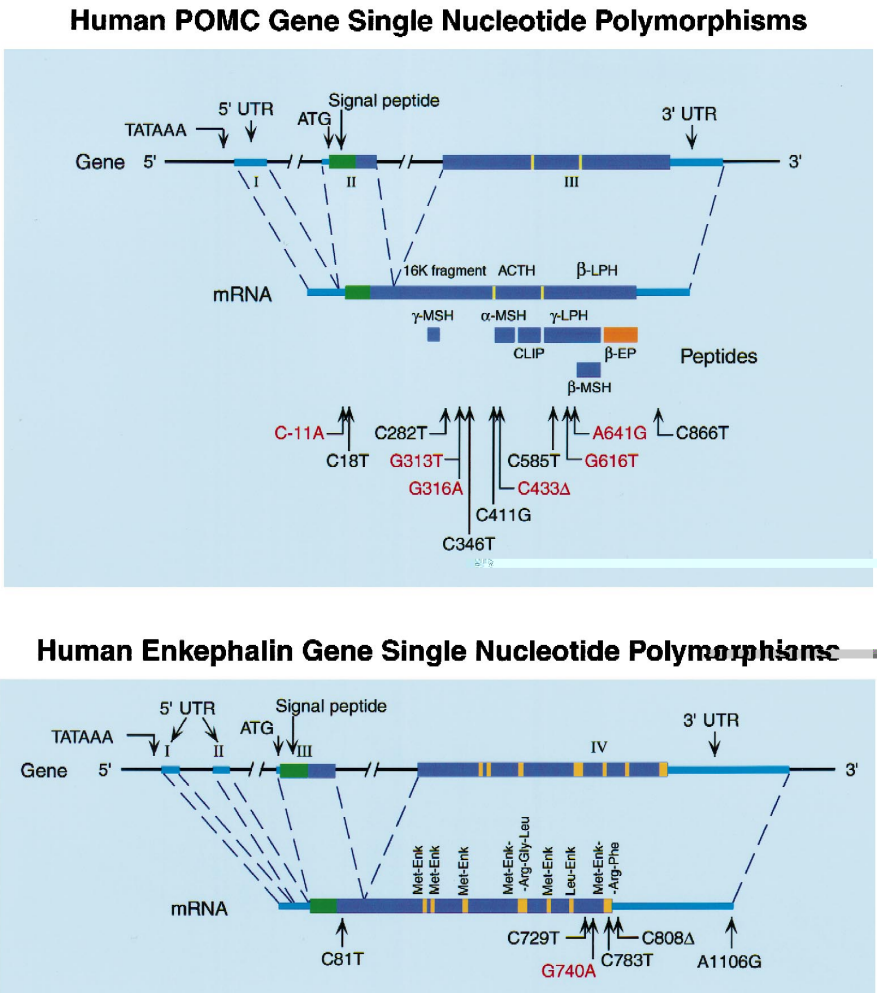

Fig. 2. The human proopioimelanocortin (POMC) and enkephalin genes, peptides, and positions of identified single nucleotide substitutions and single nucleotide deletions. The three exons of the POMC gene and four exons of the enkephalin genes are shown, with positions of 5′ and 3′ untranslated regions, signal peptide, and derived bioactive peptides indicated. Positions of the TATAA promoter element and ATG initiation codon are shown. Positions of identified coding region single nucleotide substitutions and single nucleotide deletions are indicated. Substitutions that alter the predicted primary structure of the prepropeptide are in red, as is one noncoding substitution (C-11T of the POMC gene) which has been shown to suppress translation of the gene product. (For literature citations of currently identified single nucleotide polymorphisms, see Tables 4 and 5.) Abbreviations: UTR, untranslated region; ACTH, adrenocorticotropic hormone; LPH, lipotropin; MSH, melanocyte stimulating hormone; CLIP, corticotropin-like intermediate lobe peptide; β-EP, β-endorphin.

associations of this polymorphism (Chan et al., 1994; Comings et al., 1999).

In a study of 150 schizophrenic subjects, several single nucleotide polymorphisms in the enkephalin gene were identified using dideoxy fingerprinting or single strand conformation polymorphism analysis and DNA sequencing (Mikesell et al., 1996). In this study, promoter region sequences, the entire coding region (including intron/exon boundaries), and 3′ untranslated region sequences were screened. Seven polymorphisms were identified, including six single nucleotide substitutions and a single base deletion; these are listed in Table 5 and indicated in Fig. 6. None of these polymorphisms change the predicted amino acid sequence of the propeptide. However, an additional single nucleotide polymorphism (G724A) that leads to a predicted amino change (Gly247Asp) in the coding region was identified in one schizophrenic subject. This change results in a non-conservative substitution of an amino acid residue that, while not in the sequence coding for one of the seven enkephalin peptides, is at a site completely conserved among human, bovine, rat, mouse, toad, and guinea pig genes. Screening of additional African American subjects (46 schizophrenics and 40 controls) failed to identify any additional individuals with this variant. In a subsequent report, this missense polymorphism was not detected in 67 schizophrenic and 720 control individuals (Mikesell et al., 1997).

2.6. Polymorphisms in the human dynorphin gene

Two restriction fragment length polymorphisms (one at a *Taq* I and the other at an *Msp* I site) were identified in the preprodynorphin gene by Litt et al. (1988). These two polymorphisms were found in complete linkage disequilibrium in 37 unrelated individuals, which suggests that the

Table 5
Single nucleotide substitutions and single nucleotide deletions in the human enkephalin and dynorphin genes

Nucleotide substitution[a]	Location in gene[b]	Amino acid substitution[a]	Functional domain	Detection method[c]	Overall allelic frequency reported
(A) Human enkephalin gene					
IVS1 C28A	Intron 1	NA	5′ untranslated region	A, B, E	0.405[d]
C81T	Exon III	Synonymous (Ser27)	Propeptide	A, B, E	0.089[d]
C729T	Exon IV	Synonymous (Ser243)	Propeptide	A, B, E	0.020[d]
G740A	Exon IV	Gly247Asp	Propeptide	A, B, C	< 0.01[d,e]
C783T	Exon IV	Synonymous (Tyr261)	Met-enkephalin-Arg-Phe	A, B, E	0.020[d]
C808Δ	Exon IV	NA	3′ untranslated region	A, B, E	0.392[d]
A1106G	Exon IV	NA	3′ untranslated region	A, B, E	0.266[d]
(B) Human dynorphin gene					
G-377A	5′ flanking	NA	Putative promoter	A	0.432[f]

Abbreviations: NA, not applicable.

[a] Numbering system for single nucleotide polymorphisms and amino acids is as defined in Table 2. Prototype sequences used in numbering: enkephalin, Comb et al. (1983); dynorphin, GenBank accession number X02536.

[b] Intron/Exon organization for the enkephalin gene from Comb et al. (1983).

[c] Detection methods used in the cited report: (A) sequencing of PCR amplified DNA, (B) single strand conformation polymorphism analysis, (C) PCR-restriction fragment polymorphism analysis, and (E) heteroduplex analysis.

[d] Mikesell et al. (1996).

[e] Mikesell et al. (1997).

[f] Geijer et al. (1997).

restriction fragment length polymorphisms represent an insertion/deletion rather than independent site polymorphisms. The reported population frequency of these variants in this study was 0.03. In a study of 70 chronic alcoholics and 55 control subjects, Geijer et al. (1997) used DNA sequencing of PCR amplified sections of the putative promoter region, exon I, and intron A, as well an exon IV region, which encodes the opioid peptides. One single nucleotide polymorphism, G-377A has been identified in the dynorphin gene, with an overall allelic frequency of 0.436. This position is suggested to be within the promoter region of the gene (Geijer et al., 1995).

In their initial report of the cloning of the human dynorphin gene, Horikawa et al. (1983) identified a 68 base tandem repeat later shown to be upstream from the putative transcription start site (Geijer et al., 1995). Recently, this tandem repeat has been identified as a four allele polymorphism (Zimprich et al., 2000). This study was conducted in 118 subjects with heroin addiction and 111 unaffected control subjects. The repeat element was identified by sequencing PCR amplified DNA and found to occur in a single copy or tandemly repeated either two, three, or four times. These alleles were defined as alleles 1 to 4, for the number of repeats contained, and had observed overall allele frequencies of 0.015, 0.297, 0.673, 0.015 for alleles 1 to 4, respectively. This polymorphism is of particular interest, since specific alleles have potential functional differences (see below).

3. Population distributions of human opioid system gene alleles

Allelic frequencies of specific single nucleotide polymorphisms and other polymorphisms of the human opioid system genes have been shown to vary across populations. This is not an unexpected finding, since it is well know that specific allele frequencies often vary in different populations (e.g. Lin et al., 1994; Chu et al., 1998; Cargill et al., 1999; Halushka et al., 1999). Studies in diverse populations and ethnic groups have been described for specific common alleles of two of the opioid system genes, the human μ-opioid receptor and the enkephalin genes; an examination of these studies will serve to illustrate this point.

3.1. Distributions of human μ-opioid receptor gene alleles

The two common coding region single nucleotide polymorphisms (C17T and A118G) and a (CA)n repeat polymorphism of the μ-opioid receptor gene have been studied in several ethnically, culturally, and geographically distinct populations. In evaluating these reports, it is important to note the techniques used, since, as discussed above, some methods may systematically fail to detect specific polymorphisms.

Berrettini et al. first identified the C17T single nucleotide polymorphism of the μ-opioid receptor gene and reported its overall frequency as 0.16 in a cohort that contained 51 Caucasian Americans and 54 African Americans. The authors reported that no difference was observed in allele frequencies between the two ethnic groups. This finding is in sharp contrast with subsequent reports of population frequencies of this allele. For example, Bergen et al. (1997), studying American and Finnish Caucasians and Southwest Native Americans, found this allele in two of 134 chromosomes examined (0.015 allelic frequency). We studied three ethnic/cultural groups in New York, and

found highly significant differences in allele frequencies for the C17T variant among these three ethnic groups ($\chi^2 = 26.0$, $P = 2 \times 10^{-6}$); observed allele frequencies are shown in Table 6 (Bond et al., 1998). Gelernter et al. (1999) also studied this allele in several ethnic groups. Differences in allele frequencies were found to be highly significant ($\chi^2 = 48.7$, $P < 10^{-4}$). In a recent study, Li et al. (2000), using PCR restriction fragment length polymorphism assays, did not observe the C17T substitution in 540 subjects from Sichuan Province, China.

The A118G single nucleotide polymorphism in the μ-opioid receptor gene coding region has also been studied in several distinct populations; allele frequencies are listed in Table 6. The first group to identify this variant reported relatively similar overall allele frequencies in the populations they studied (Bergen et al., 1997). We found significant differences in frequency of this the A118G variant among ethnic groups we studied ($\chi^2 = 7.15$, $P = 0.028$) (Bond et al., 1998). Gelernter et al. (1999) also reported highly significant differences in allelic frequencies across several populations for this variant ($\chi^2 = 69.3$, $P < 10^{-4}$). Li et al. (2000), studying Han Chinese also reported this allele at a high overall frequency (0.321) in that population.

Gelernter et al. (1998) studied the frequencies of the μ-opioid receptor (CA)n repeat polymorphism in two ethnic groups living in Connecticut (African American and European American), and DNA isolated from stored lymphoblastoid cell lines derived from several indigenous populations, including one specific Eastern European population (Adygei), two native American populations, (Maya, from the Yucatan peninsula, and Rondonian Surui, from the Amazon basin), one Asian population (Chinese), and one population from sub-Saharan Africa (Mbutu Pygmies). In this study, nine separate alleles were described using PCR amplification and acrylamide gel electrophoresis. Highly significant differences in allele frequencies were observed across populations (with the seven least common alleles pooled into a single group, $\chi^2 = 137.7$, $P < 10^{-6}$). Highly polymorphic repeat alleles such as these might be expected to show large differences in allelic frequency, depending on their age and history in the population, and also depending on whether they hold any selective advantage or disadvantage or are in linkage disequilibrium with an allele on which selection could act.

3.2. Distributions of human enkephalin gene alleles

The frequency of alleles of the (CA)n repeat in the 3′ untranslated region of the preproenkephalin gene was studied in several ethnically, culturally or geographically isolated populations including Chinese and Atayal living in Taiwan, Caucasians and African Americans living in the United States, and Caucasians living in Byelorussia, (Chan et al., 1994). In this study, no significant differences in allele frequencies were observed between the Chinese and Ayatal populations, or between Caucasians living in the United States or Byelorussia. However, highly significant differences were found in pairwise comparisons between the Chinese, Caucasian, and African American samples.

Distributions of several single nucleotide polymorphisms of the preproenkephalin gene were also found to vary significantly among Caucasians of Northern or Western European descent and African American study subjects (Mikesell et al., 1996). A small number of Asian and Native Americans were also included in this study, although the sample sizes of these two groups were too small to evaluate differences in allele frequencies. Two single nucleotide polymorphisms, which were observed in complete linkage disequilibrium, were found only in the African American group. Three other polymorphisms (two single nucleotide substitutions and a single base deletion) were found primarily in the Caucasian group. Interestingly, two single nucleotide polymorphisms were observed to be in complete linkage disequilibrium in the Caucasian and Native American populations but not in the African American group.

As illustrated above, alleles of the opioid system genes vary among populations of different ancestry, defined (in

Table 6
Allelic frequencies of the variant allele of C17T and A118G single nucleotide polymorphisms of the human μ-opioid receptor gene in diverse populations

Ethnicity or population	C17T		A118G		
	Bond et al. (1998)	Gelernter et al. (1999)	Bergen et al. (1997)	Bond et al. (1998)	Gelernter et al. (1999)
European American (Caucasian)	0.019 (52)	0.008 (470)	0.105 (100)	0.115 (52)	0.141 (543)
African American	0.210 (31)	0.140 (143)		0.016 (31)	0.028 (144)
Hispanic	0.037 (67)	0.011 (46)		0.142 (67)	0.117 (47)
Southwest Native American			0.163 (367)		
Finnish Caucasian			0.122 (324)		
Ethiopian		0.080 (49)			0.170 (49)
Bedouin		0.050 (43)			0.080 (43)
Ashkenazi		0.016 (93)			0.210 (93)
Japanese		none observed (35)			0.485 (34)

Allele frequency for the variant allele is shown for various study populations. Numbers in parentheses are the number of subjects in whose genotype was ascertained in each study. A study of Han Chinese found the 118G allele at a frequency of 0.321, and no occurrence of the 17T allele in 540 subjects (Li et al., 2000).

these reports) by ethnicity or by geographic origin. The patterns of allele distributions are complex and specific to each polymorphism. Since stratification of alleles within study populations can lead to spurious associations between candidate markers and specific phenotypes, it is important to take into consideration the potential effects of population admixture in association studies. Grouping by ethnicity is, at best, an imperfect method for inferring ancestry within structured populations; however, it is one means by which problems introduced into association analyses by admixture can be reduced. Increasing globalization and migration of human populations is increasing the complexity of using ethnic classification as a determiner of genetic relatedness. At the same time, since specific polymorphisms may be unique to specific populations, or have very different allele frequencies, it is also important to survey diverse populations for the discovery of polymorphisms, including single nucleotide polymorphisms, and for studies of their potential associations with disease or other phenotypes.

4. Endogenous opioid system gene polymorphisms and addictions

4.1. μ-Opioid receptor gene polymorphisms and addictions

The human μ-opioid receptor gene has been a primary focus of studies of the potential association of endogenous opioid system genes with alcohol and drug abuse or dependence. With one exception (Bergen et al., 1997), all studies reported to date have used a classical case control association study design.

Berrettini et al. (1997) studied individuals with either opioid or cocaine dependence and matched control subjects. Research diagnostic criteria (RDC) diagnoses of opioid or cocaine dependence were established through a review of medical records by a psychiatrist. In this cohort, no significant differences with respect to allele frequencies of the C17T and G-38T single nucleotide polymorphisms were observed between opioid and cocaine dependent groups, so they were combined and compared with controls. No differences in allele frequency of the G-38T substitution were observed between cases (combined drug dependence) and controls (no drug dependence). In contrast, a higher frequency of the 17T allele was observed in the drug dependent group compared to controls at a borderline significance level ($\chi^2 = 4.1$, $P = 0.05$), although the authors state in this report that this significance level is below that recommended for statistical significance in association studies (e.g. see Kidd, 1993).

Bergen et al. (1997) studied the potential association of two single nucleotide polymorphisms of the μ-opioid receptor gene (A118G and IVS2 C691G) and alcohol dependence with or without other drug dependence (DSM-III-R criteria). The authors used a case control design to study these alleles in American and Finnish Caucasians and Southwestern Native Americans and also studied sib-pair linkage in the Native American population. No significant association or linkage between the A118G or IVS C691G alleles, genotypes, or haplotypes to any disorder category was observed.

We evaluated differences of allelic frequencies of the A118G and C17T variants in study subjects with opioid dependence (with or without other drug or alcohol abuse or dependence) and control subjects with no history of drug or alcohol abuse or dependence (Bond et al., 1998). To ensure that all individuals in the opioid dependent group were severely affected by the disorder, study subjects in this group all met the federally-regulated criteria for admission into a methadone maintenance treatment; these criteria are significantly more stringent, particularly with respect to frequency of use (multiple daily) and duration of such use (more than 1 year), than many psychiatric diagnostic criteria commonly used in studies of this type (e.g. DSM-III-R or DSM-IV). Control subjects had no current or prior period of extended alcohol or drug abuse. With all ethnic groups combined, we found a trend towards a higher proportion of the 17T allele in individuals with opioid dependence ($\chi^2 = 3.70$, $P = 0.054$), similar to the report of Berrettini et al. (1997). Also, with data stratified by ethnicity, the pooled relative risk was calculated for this allele and Mantel–Haenszel Chi-square test used to evaluate differences in relative risk between cases and controls. Again, a borderline significance level was found with the relative risk greater for individuals carrying the 17T allele ($\chi^2 = 3.73$, $P = 0.050$). With respect to the even more frequent A118G polymorphism, with all ethnic groups combined, no differences in allele frequency or pooled relative risk were found to be significant. However, within one ethnic/cultural group (Hispanic Americans), we found the 118G allele in a significantly higher proportion of control individuals compared to opioid dependent subjects, similar to the findings of Berrettini et al. (1997). This finding is most likely due to population stratification or admixture within this study group; however, it also suggests the possibility that this allele might confer a relative protection against opioid addiction. This finding is particularly intriguing, in view of the potential functional difference of this allelic variant (see below).

The studies of Sander et al. (1998) and Town et al. (1999) further addressed the possible association of the A118G polymorphism with alcohol dependence. In a study of 327 alcohol dependent subjects and 340 control subjects, with all study subjects of German ancestry, no differences in A118G allele frequencies were observed between alcohol dependent cases (ICD-10 criteria) and controls (Sander et al., 1998). Control subjects were unscreened blood donors without psychiatric assessment. An allele specific PCR assay was used for genotyping. In this study, three endophenotypes within the alcohol dependence category were also defined to select alcoholics with

a presumed high genetic load. These endophenotypes were a family history of parental alcoholism ($n = 114$), onset of inability to refrain from alcohol use before age 26 ($n = 73$), a history of alcohol withdrawal seizure or delirium ($n = 107$). No significant differences in allele frequencies between any of these specific endophenotypes and controls were observed. Like the study of Bergen et al. (1997), this study found no evidence for involvement of the A118G polymorphism and alcohol dependence. However, one potential problem with the study design in this report is that control subjects were unscreened anonymous blood donors. It is well established that persons with chemical dependence are in a significantly higher proportion of individuals seeking to donate blood compared to persons with no history of chemical dependence. Therefore, since in this study control individuals were not assessed with respect to addictive disease status, the "control" samples may have inadvertently included a significant proportion of individuals with chemical dependence.

In contrast with the previous study, Town et al. (1999) reported a positive association of the μ-opioid receptor A/A genotype at position 118 of the μ-opioid receptor gene and alcohol dependence. One hundred and five alcohol dependent individuals (DSM-IV criteria) were compared to 122 control individuals. The subjects were primarily Caucasian (81.9%) from either the Tampa or Miami areas of Florida. Genotyping was performed by PCR-restriction fragment length polymorphism assays (Bergen et al., 1997). No differences in genotype or allele frequencies were observed between Caucasian and non-Caucasians, which might have been be due to small numbers in the non-Caucasian sample. The authors combined the Caucasian and non-Caucasian groups in subsequent analyses. Alcohol dependent cases were compared to controls with respect to A118G genotype and allele frequencies. Both the A/A genotype and A118 allele had significantly higher frequencies in the alcohol dependent group. Odds-ratio calculations showed that in this study population, A/A homozygotes had a greater than two-fold risk for alcohol dependence, and the A118 allele carried an approximate two-fold risk for the disorder. This study, therefore, supports the hypothesis that the prototype A118 allele might confer a relative risk for alcohol dependence, and the 118G allele, therefore, a relative protective effect.

In a recent study, Gelernter et al. (1999) reported on associations of the A118G and C17T polymorphisms with alcohol and drug dependence (DSM-III-R criteria). Alcoholic subjects were all Caucasian; control and drug dependent subjects included Caucasian, African American and Hispanic individuals. All comparisons by diagnosis were made within ethnic classifications. No significant differences in allele frequencies for the A118G or C17T polymorphisms with respect to diagnosis were observed.

An additional study of Han Chinese by Li et al. (2000) also found no evidence for an association of heroin abuse and the A118G polymorphism. In this study, 282 individuals meeting DSM-IV criteria for heroin abuse were compared to 258 control individuals recruited from staff, students, and acute medical inpatients in a general care hospital. Control individuals did not undergo psychiatric evaluation, but were asked if they had ever been admitted to a hospital, prescribed medication or told by a doctor that they had a mental or neurological illness; such subjects were excluded. No differences were observed in allele frequencies for either the A118G or IVS2 C691G variants in opiate abuse subjects compared to controls. As noted above, this study population had a higher frequency of the 118G allele than other ethnic groups studied; the 17T allele was not observed in this population.

One other polymorphism of the μ-opioid receptor gene, the (CA)n repeat, has been studied with respect to alcohol or other drug dependence (Kranzler et al., 1998). In this study, individuals with a primary diagnosis (DSM-III-R criteria) of dependence on either alcohol ($n = 240$), cocaine ($n = 154$), or opioids ($n = 33$) were compared with 118 control subjects. Approximately half the control subjects were rigorously screened to exclude substance abuse disorders, the other half were unscreened. Analysis of allele frequency differences was conducted with data stratified by ethnic group. In the Caucasian group, there was evidence of a significant association of allele frequency with all substance abuse groups combined (Likelihood ratio test (LTR) $\chi^2 = 3.52$, $P = 0.030$). However, this significant finding disappeared when data were analyzed separately for specific substances. In the African American group, no significant differences in allele frequency distributions were observed comparing controls with all substance abuse groups combined or between controls and each substance abuse category separately. After making corrections for multiple comparisons, the authors suggest that these data do not support the hypothesis that alleles at this locus are associated with drug or alcohol dependence.

In summary, some studies have provided support for the hypothesis that specific alleles of the μ-opioid receptor gene are associated with alcohol, opiate or other drug dependence, whereas other studies have not. The majority of genetic association studies reported to date have evaluated the common single nucleotide polymorphisms in the N-terminal domain of the receptor, which lead to potentially functional amino acid changes in the predicted primary structure of the receptor. Existing evidence with respect to specific addictive diseases supports either no effect or a protective effect for the 118G allele and either no effect or an enhanced vulnerability for individuals carrying the 17T allele. Further studies in diverse well-characterized populations are necessary.

4.2. δ-Opioid receptor gene polymorphisms and addictions

The possible role of the T921C single nucleotide polymorphism of the human δ-opioid receptor gene in susceptibility to heroin and alcohol dependence has also been

studied. Although this substitution is synonymous, it was found at a high allelic frequency in the study group (all individuals of German origin), and therefore has potential utility as a genetic marker. In an initial report, the rarer 921C allele was found in a significantly higher proportion of heroin dependent individuals ($n = 103$) than in control subjects ($n = 115$) (Mayer et al., 1997). Heroin dependent subjects were either individuals hospitalized for heroin dependence, persons who died of heroin overdose and who had a documented history of heroin abuse, or individuals who were diagnosed as heroin dependent by psychiatric examination. In this study, the higher allelic frequency of the 921C allele was due to a higher proportion of C/C homozygotes in heroin dependent subjects. An attempt to replicate this finding in a case control association study design was subsequently performed, and also the transmission of the 921C allele to affected offspring (Franke et al., 1999). In the case control study of 333 heroin dependent subjects, 262 alcohol dependent subjects (heroin and alcohol dependence defined by DSM IV criteria), and 173 control subjects, no differences in genotype or allele frequencies between patient groups and controls. Also, no preferential transmission of the 921C allele was observed in either 90 heroin or 72 alcohol dependent subjects. This study, therefore, does not support a role for the T921C polymorphism in either alcohol or heroin dependence. However, as noted above for the report of Sander et al. (1998), these studies used unscreened anonymous blood donors for control subjects, and the control group may have included persons with chemical dependence.

A preliminary report on an additional coding region δ-opioid receptor single nucleotide polymorphism (T82G), which causes an amino acid substitution in the N-terminal domain of the receptor (Phe27Cys), found no association of this polymorphism with heroin addiction (Höllt in LaForge and Kreek, 2000).

4.3. Enkephalin gene polymorphisms and addictions

The possibility of an association of alcoholism and alleles of the (CA)n repeat element in the 3′ untranslated region of the enkephalin gene was studied by Chan et al. (1994). As noted above, allelic frequencies were found to vary significantly among ethnic groups, therefore, differences in allele frequencies were evaluated in data stratified by ethnicity. Diagnostic methods differed for each ethnic group: in Chinese and Atayal groups psychiatrists evaluated subjects using DSM-III-R diagnostic criteria; Caucasian and African American alcoholic subjects were recruited from alcoholism treatment programs, approximately 50% of Caucasians and 67% of African American alcoholic subjects also had clinical alcoholic organ pathology. Control subjects were non-alcoholics recruited for population studies; however, procedures for evaluation of presence or absence of addictive diseases were not reported for these subjects. No significant differences in allele frequencies were observed between alcoholic and control subjects by contingency table analysis.

In a second study, allele frequencies of the (CA)n repeat element were determined in 31 opioid dependent subjects, 89 subjects who had primary dependencies on alcohol, cocaine, or amphetamine, and 132 control subjects (Comings et al., 1999). Addictive disease subjects were recruited from an addiction treatment program and DSM-III-R diagnoses made using structured and semistructured instruments by trained interviewers. Control subjects were university students. All individuals were non-Hispanic Caucasians. In this study, two common alleles accounted for 98.75 of the total alleles. Two rare alleles were also found; in order to include them in the analysis, they were grouped with one or the other common alleles according to length. Significant differences in allele and genotype frequencies were observed between opioid dependent subjects and either control subjects or subjects with non-opioid substance dependence, with the longer alleles more common in opioid dependent subjects. The authors also compared control subjects with published allele frequencies in Caucasian control subjects from two previous reports (Weber and May, 1990; Chan et al., 1994) and found no differences in allele frequencies. Control subject allele frequencies from their study and from literature reports were pooled and compared with opioid dependent subjects; again, a significant association of the longer alleles with opioid dependence was found ($\chi^2 = 7.01$, $P < 0.008$). Evidence from this study supports the hypothesis that alleles of this gene may be associated with opioid dependence. However, it should be noted that the number of opioid dependent subjects was small ($n = 31$), and this finding may be due to sampling error.

4.4. Dynorphin gene polymorphisms and addictions

The potential association of alcoholism and the G-377A single nucleotide polymorphism in the 5′ flanking (and putative promoter) region of the dynorphin gene has been studied in 70 in-patient alcoholic subjects and 55 matched control subjects (Geijer et al., 1997). Sixty of the alcoholics were men and 10 were women; 47 of the control subjects were men and eight were women. All subjects were of Swedish or Finnish origin. Alcoholic subjects were stratified by age of onset (onset of drinking before the age of 25) and presence or absence of alcoholism among parents. A significantly higher rate of parental alcoholism was found in probands with early onset of drinking. No significant differences in genotype distributions were found between control subjects compared with either total alcoholic subjects, late onset alcoholic subjects, or alcoholics with no alcoholism among parents. In addition, no differences in genotype distributions between Finnish and Swedish subjects were found. When the comparisons between alcoholics and controls were made within only the Swedish group, again, no significant differences were

found. This study, therefore, provides no evidence for an association of this polymorphism and alcoholism.

The possible implications for heroin addiction of a potentially functional 68 base polymorphic repeat in the putative promoter region of the human dynorphin gene has also been investigated in a recent study (Zimprich et al., 2000). The subjects studied were the same cohort of 118 heroin addicts and 111 controls used in a previous study of association of heroin addiction with alleles of the δ-opioid receptor gene described above (Mayer et al., 1997). Four separate alleles were described based on the number of 68 base repeats present. No significant differences in allele frequencies between heroin addicts and control subjects was observed in this cohort (Zimprich et al., 2000).

5. Functional studies of polymorphisms in human opioid system genes

5.1. Potential functional polymorphisms in the human μ-opioid receptor gene

The effects of polymorphisms in the human μ-opioid gene on receptor function has been studied in both in vitro assay systems and in clinical association studies with specific aspects of physiology that may have importance in addictions and other diseases.

In the first such study, we investigated the most frequent coding region variant identified to date, the A118G single nucleotide polymorphism, which results in the substitution of the amino acid asparagine to aspartic acid at amino acid position 40 (Asn40Asp or N40D) (Bond et al., 1998). This substitution alters a putative N-linked glycosylation site in the N-terminal domain of the receptor, a region which has been implicated in determining binding affinity of the μ-opioid receptor to specific ligands (Chaturvedi et al., 2000). We studied binding and cellular activity of the N40D variant receptors compared to the prototype receptor in two cellular systems (AV-12 cells, which are a cell line derived from Syrian hamster, and *Xenopus* oocytes) both of which are known to glycosylate transcribed proteins. In membranes prepared from AV-12 cells stably expressing either the prototype or N40D variant receptors, we observed most exogenous and endogenous ligands of the μ-opioid receptor showed no difference in binding affinity (Bond et al., 1998). These ligands included the small endogenous peptide agonists Met- and Leu-enkephalin, endomorphin-1 and-2; the μ-selective synthetic opioid peptide [D-Ala^2, *N*-methyl-Phe^4, Gly-ol^5]enkephalin (DAMGO); dynorphin A-(1–17), which is the natural endogenous ligand of the κ-opioid receptor; the μ-preferring opioid alkaloid agonists morphine, fentanyl, and methadone; and the opioid antagonist naloxone. Unexpectedly, the 31 amino acid neuropeptide β-endorphin bound the variant receptor approximately three times more tightly than the prototype receptor (Bond et al., 1998).

We also studied the N40D variant receptor in a second in vitro assay to evaluate an important intracellular function of the μ-opioid receptor, agonist induced inhibition of neuronal excitability mediated by inhibition of presynaptic Ca^{2+} channels and activation of postsynaptic K^+ channels (Bond et al., 1998). The primary effector K^+ channels for the μ-opioid receptor are G protein-activated inwardly rectifying K^+ channels. *Xenopus* oocytes were injected with in vitro transcribed RNA of either the prototype or N40D variant receptor as well as the RNAs of G protein-activated inwardly rectifying K^+ channels and agonist-induced K^+ currents measured. Agonist stimulation with the μ-selective peptide endomorphin-1 evoked similar K^+ currents for the prototype and N40D variant. However, β-endorphin activated the N40D variant receptor with three times more potency than it activated the prototype receptor. This greater activation is consistent with the difference in binding affinity of β-endorphin to the variant receptor (Bond et al., 1998).

β-endorphin is the most protean of the endogenous opioid peptides; it is expressed in the brain, pituitary, and peripheral tissues. It is a primary endogenous ligand of the μ-opioid receptor, the longest endogenous opioid receptor ligand, and the endogenous opioid receptor ligand with the longest half-life. Centrally, it plays a role in antinociception. Peripherally, (and possibly centrally as well), β-endorphin is an important mediator of diverse physiologic functions including the hypothalamic–pituitary–adrenal axis mediated response to stress, of particular importance to addiction, since variation in stress responsivity has been postulated to be mechanistically involved in specific addictive diseases (Kreek et al., 1981; Kreek, 1996a,b; Culpepper-Morgan and Kreek, 1997; Kreek and Koob, 1998). The observed differences in binding and cellular activation of the μ-opioid receptor by β-endorphin may therefore contribute to individual variability in endogenous physiology, including those of importance to specific addictions.

Additional evidence that the N40D variant μ-opioid receptor may have consequences important for physiologic function and responses to pharmacotherapy are provided by two human studies of association of specific phenotypes to the genotype at the A118G polymorphism.

In a study of alcoholics at two time points following withdrawal, Smolka et al. (1999) measured apomorphine-induced growth hormone secretion as an indirect indicator of central dopaminergic sensitivity. Homozygous individuals carrying the prototype A/A genotype at position 118 were compared to A/G heterozygotes. The authors observed marginal differences with respect to genotype in apomorphine-induced growth hormone responses both before detoxification and after 3 months of abstinence. In contrast, growth hormone responses measured at an intermediate time point (7 days after alcohol withdrawal) were significantly higher in individuals carrying the heterozygous A/G genotype compared to A/A homozygous individuals.

In a preliminary report of a second study, Strong et al. (1999) ascertained the A118G genotypes of Parkinson Disease patients treated with the antiparkinson medication levodopa (L-DOPA). Subjects heterozygous for the A118G polymorphism were significantly more likely to develop dyskinesia after less than 5 years of treatment, compared to individuals who progressed to dyskinesia after five or more years or not at all. The number of subjects in this study was small ($N = 26$), and the conclusions must therefore be regarded as preliminary, however, both this study and that of Smolka et al. (1999) raise the intriguing possibility that the N40D variant encoded by the A118G polymorphism may alter the usual μ-opioid receptor mediated dopaminergic response and functioning, with potentially important implications for clinical treatment. Both studies included individuals heterozygous for the A118G polymorphism; no G/G individuals homozygous for the variant receptor were studied. Since heterozygous individuals are likely to express both receptor variants and be intermediate in any phenotypic differences caused by the variant forms, it would be of interest to identify individuals homozygous for each variant receptor in future studies of such μ-opioid receptor–dopamine system interactions, as well as other aspects of μ-opioid receptor mediated physiological function.

An association study of the A118G genotype and idiopathic absence epilepsy was reported by Sander et al. (2000). They observed significantly higher frequency of the variant 118G allele in subjects with this disorder compared to matched controls. Several other studies have suggested that the μ-opioid receptor may be involved in the etiology or expression of this disorder, and again, the genotype at the A118G polymorphism may be useful as a predictor of individuals with increased liability of epileptogenesis.

In a preliminary report, Befort, Kieffer et al. (in LaForge and Kreek, 2000) also used in vitro assays to study potential functional differences in human μ-opioid receptor variants encoded by several other polymorphic alleles of the gene. The N40D variant was studied as well as receptors with amino acid substitutions Asn152Asp (N152D) in the third transmembrane domain, and Arg265His (R265H) and Ser268Pro (S268P) in the third intracellular loop. These variant receptors result from single nucleotide polymorphisms in the gene (as reported by M. Hoehe, see Befort et al. in LaForge and Kreek, 2000). Receptors containing the amino acid substitutions were expressed in COS cells, (a transformed cell line derived from African Green Monkey kidney), and compared to cells expressing the prototypic receptor. The N152D variant was found to be expressed at lower levels than the prototype or other variant receptors, although possible mechanisms for this were not explored and potential physiological implications of this phenomenon remain unclear. The authors also observed increased binding of the synthetic peptide agonist DAMGO, the synthetic peptide antagonist D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP) and the alkaloid antagonist diprenorphine to the R265H variant compared to prototype receptor (3.7-, 1.9-, and 2.5-fold, respectively). Also, binding of morphine was observed to be greater at several variant receptors (reported as a range from 2.6- to 4-fold, not specific with respect to which variant). Bond et al. (1998) found no difference in binding of morphine to the N40D variant compared to the prototype. Befort, Kieffer et al. (in LaForge and Kreek, 2000) also investigated receptor signaling as measured by an agonist-induced [^{35}S]GTPγS binding assay. Interestingly, a dramatic impairment of agonist efficacy was observed for the S268P variant receptor. Agonist potency at other variants was not significantly different from the prototype. These data suggest that the S268P variant may show a significant loss of receptor function, which could affect opioid-regulated physiology or behaviors (Befort et al. in LaForge and Kreek, 2000).

Two reports also studied potential functional consequences of the S268P variant (originally identified by M. Hoehe reported by Befort et al. in LaForge and Kreek, 2000) of the μ-opioid receptor (Höllt, reported in LaForge and Kreek, 2000; Koch et al., 2000). These authors point out that Ser268 is a putative phosphorylation site for calcium/calmodulin-dependent protein kinase II (CamKinase II). In oocyte assays, the S268P receptor was found to have a loss of CamKinase II induced receptor desensitization as measured by functional coupling to co-expressed G protein activated inwardly rectifying K^+ channels. In similar assays, a significant reduction in agonist induced receptor desensitization was also observed. In human embryonic kidney (HEK) cells stably transfected with the S268P or prototype receptor, the binding affinity of the peptide agonist DAMGO was similar. However, maximal agonist induced inhibition of forskolin-stimulated adenylyl cyclase activity was significantly reduced in the S268P variants. Also, the time course of agonist induced receptor desensitization, as measured by adenylyl cyclase activity, was slower for the S268P variant. Finally, similar to the report by Befort et al. (in LaForge and Kreek, 2000), the authors observed a reduction in functional coupling of the S268P variant using [^{35}S]GTPγS binding assays. Taken together, these studies point to potentially significant functional consequences for individuals carrying this polymorphism. However, this variant, which is located in the third exon of the receptor, has not been identified in other studies that scanned for allelic variation in this receptor, and its occurrence may be rare. The potential significance of this single nucleotide polymorphism in contributing to vulnerability to addictive diseases may therefore be limited.

5.2. Functional studies of the human δ-opioid receptor variants

One preliminary report on potential functional human δ-opioid receptor gene polymorphisms found no signifi-

cant effects of the predicted amino acid substitution (Phe27Cys) caused by the T80G single nucleotide polymorphism on binding and coupling of β-endorphin or other δ-opioids measured either in *Xenopus* oocytes or HEK293 cells transfected with the variant forms of the receptor (Höllt in LaForge and Kreek, 2000).

5.3. POMC gene variants and functional variation

Several polymorphisms that disrupt expression of POMC gene products have been demonstrated to have significant physiological and pathological consequences. As noted above, Krude et al. (1998) identified two single nucleotide substitutions and a single base deletion (C-11A, G313T, and C433Δ), which, in a homozygous or compound heterozygous condition, result in near or complete deficiency of POMC derived peptides, leading to a phenotype of red hair pigmentation, adrenal insufficiency, and severe early-onset obesity. Other polymorphisms predicted to disrupt production of POMC gene products have also been identified (Hinney et al., 1998). Although these variants were not found to be common, they underscore the possibilities of intrinsic physiological differences caused by polymorphisms at this gene. Although no phenotype caused by deficiency in production of the opioid peptide β-endorphin was reported, aspects of physiology under control of this peptide were not reported in this study (Krude et al., 1998).

Some similarities and connections involving the endogenous opioid system have been noted between eating disorders and addictive disorders. Several polymorphic variants of the POMC gene have also been studied in individuals with obesity or anorexia nervosa (Hinney et al., 1998; Echwald et al., 1999). In these studies, no ready association was observed between any POMC polymorphisms and phenotypes measured, including mean body mass index and circulating leptin levels. However, unlike the study by Krude et al. (1998), no individuals studied were homozygous or compound heterozygous for polymorphisms predicted to disrupt POMC gene expression. A third study has identified a statistically significant association of specific haplotypes of allelic variants of the POMC gene with variation in circulating leptin levels (Hixson et al., 1999).

5.4. Potential functional polymorphisms in the human dynorphin gene

The potential functional importance of the 68 base tandem repeat polymorphism in the human δ-opioid receptor gene promoter region was studied by Zimprich et al. (2000). This repeat was found in one, two, three, or four copies, and contains a variant (TGACTTA) of the consensus binding site (TGACTCA) of the transcription factor complex activator protein-1 (AP-1). Using gel-shift assays, the authors demonstrated that a protein or protein complex present in NG108-15 cell nuclear extracts specifically binds to this site and binding can be competed by excess AP-1 consensus oligonucleotide, suggesting that the bound protein was indeed AP-1, and the site within the putative dynorphin promoter can bind this transcription factor. Cellular assays were also performed in NG108-15 cells transfected with constructs containing one, two, three, or four copies of the 68 base repeat linked to a reporter gene. Under basal conditions, no differences were observed in transcription levels with respect to the copy number of the repeat in each construct. However, in cells stimulated with the phorbol ester tissue plasminogen activator, which is an activator of AP-1, higher levels of transcription were observed in cells transfected with constructs of three and four copies of the repeat compared to those containing one or two copies. This suggests that individuals carrying alleles with three or four copies of the repeat might show increased levels of dynorphin mRNA transcription following specific stimuli that activate AP-1. As noted above, the authors of this study failed to find an association of alleles of this repeat polymorphism and heroin addiction. However, it would be of considerable interest to study these allelic variants in individuals with cocaine or other psychostimulant abuse, since these drugs of abuse are well known to cause sustained acute increases of prodynorphin mRNA levels in brain areas important for reward.

6. Discussion and conclusions

The terms “pharmacogenetics” and “pharmacogenomics” have come into common usage to describe genetic or genomic influences on individual variability in responses to medications used in the treatment of specific medical disorders. We have proposed the terms “physiogenetics” and “physiogenomics” to refer to the related concept of genetic or genomic influences on individual variability in physiological responses to endogenous substances such as neuropeptides or hormones (Kreek, 2000; LaForge et al., 2000a,b).

An example of a pharmacogenetic response with considerable bearing on the present discussion is provided by the gene encoding the neuronal cytochrome P450 (CYP) enzyme, CYP2D6 or sparteine/debrisoquine oxygenase. This enzyme causes the biotransformation, by *O*-demethylation, of the oral opiate analgesics codeine, oxycodone, and hydrocodone into morphine, oxymorphone, and hydromorphone, which are compounds of significantly greater analgesic potency. Approximately 5–10% of Caucasians are homozygous or compound heterozygous for defective alleles of this gene and are unable to convert codeine or its above listed congeners into their more active counterparts by *O*-demethylation (Alván et al., 1990; Tyndale et al., 1997). Consequently, individuals with geno-

types that do not allow expression the functional enzyme are prone to therapeutic failure of these analgesics (Poulsen et al., 1996). Of particular interest, individuals who lack the CYP2D6 enzyme may also be protected against oral opiate dependence (Tyndale et al., 1997).

An example of a well-defined physiogenetic response in the endogenous opioid system is illustrated by the allelic variants of the POMC gene which prevent expression of POMC-derived peptides and result in several phenotypes including at least two pathophysiologies (Krude et al., 1998). Other potential physiogenetic variants in the opioid system include the 68 base repeat polymorphism in the 5′ region of the dynorphin gene, which may cause differences in the expression of the peptide, as well as the N40D and S268P variants of μ-opioid receptor, which cause differences in binding affinity or cellular function and may have implications for physiology (Bond et al., 1998; Befort et al., 1994; Höllt in LaForge and Kreek, 2000). However, these studies were performed in cellular assay systems; further clinical studies are necessary to determine the possible effects these polymorphisms might have in integrated human physiology or behavior.

The majority of opioid system gene polymorphisms that have been identified and studied, particularly single nucleotide polymorphisms, have been in the coding regions of genes. This is not surprising, since coding region variants which cause amino acid changes could affect protein function, and are therefore of particular interest for study. Polymorphism in the promoter region, as well as in the 3′ or 5′ untranslated region can also have important effects on gene expression levels or post-transcriptional events, as is demonstrated by the G-11A single nucleotide polymorphism in the 5′ untranslated region of the POMC gene which essentially eliminates expression of the propeptide (Krude et al., 1998). Other polymorphisms, including synonymous single nucleotide polymorphisms have potential utility as genetic markers in linkage studies, particularly haplotype analyses, if their frequency in the population is sufficiently high. Also, at least theoretically, even synonymous substitutions might alter functional protein levels. The genetic code is degenerate, with all but two of the 22 amino acid residues encoded by more than one codon, and some evidence suggests that selection may act on silent site substitutions in mammals (Eyre-Walker, 1999). For example, if in some cell types levels of specific aminoacyl tRNA molecules were absent or present in low levels, genetic variants that alter specific codons, even if they encode identical amino acids, might alter translation rates, and thereby alter levels of expressed proteins. It may be postulated that some such synonymous variants or mutations might be, in fact, "quiet" rather than "silent".

To date, most human molecular genetic studies of drug addictions and many of alcoholism have used hypothesis-driven or "candidate gene" approaches. In addition, several large-scale efforts are now underway in genetic studies of specific addictions by positional methods, including the multi-site Collaborative Study on the Genetics of Alcoholism (COGA) project sponsored by the United States National Institute on Alcohol Abuse and Alcoholism, and the Human Genetics Consortium effort involving several university laboratories sponsored by the United States National Institute on Drug Abuse (see Stocker, 1998). The development of new technologies for rapid and high throughput identification of single nucleotide polymorphisms and other polymorphisms, such as microarrays ("microchips") will facilitate these studies (e.g. LaForge et al., 2000a,b). As described herein, many studies have targeted the opioid system genes because of their explicit or implicit involvement in mediating the responses to opiates and other drugs of abuse as well as alcohol. As with most disorders with a behavioral component, specific addictive diseases are phenotypically and, presumably, genetically complex disorders that are likely to be influenced by multiple alleles of multiple genes with different levels of penetrance and pleiotropy. The plasticity and redundancy of neural systems make the endeavor to pinpoint genes that contribute to vulnerability to the specific addictions particularly challenging. As in genetic studies of other complex disorders, accurate, consistent, and thorough phenotyping and endophenotyping of study subjects, including controls, is essential.

In the years since the first cloning of the opioid peptide precursors in 1979, and since the first cloning of the opioid receptors, many polymorphisms in the genes of these proteins have been identified and evaluated in genetic and functional studies. Most of these studies have been published within the past 3 years, indicating a recently increased interest by researchers in the potential importance of genetic variability in this system. Some studies have provided evidence of genetic associations of specific alleles with specific addictions; others have failed to find such associations. A few studies have provided intriguing evidence of possible functional variation in the opioid system as the result of specific polymorphisms. These findings could have broad implications for understanding human physiology and in the treatment of specific medical disorders, including addictions. Future genetic, laboratory, and ultimately, clinical studies will further elucidate the role of specific polymorphisms in the endogenous opioid system genes in specific addictions.

Acknowledgements

The authors would like to thank S. Russo, S. Fung-Ho, B. Seides, and D. Nikaj for their assistance in the preparation of the manuscript, and A. Ho for critical reading. This work was supported in part by research grants from the United States National Institutes of Health: DA00049, DA05130, DA09444, DA12848 and RR00102.

References

Akil, H., Meng, F., Devine, D.P., Watson, S.J., 1997. Molecular and neuroanatomical properties of the endogenous opioid system: implications for treatment of opiate addiction. Semin. Neurosci. 9, 70–83.

Alván, G., Bechtel, P., Iselius, L., Gundert-Remy, U., 1990. Hydroxylation polymorphisms of debrisoquine and mephenytoin in European populations. Eur. J. Clin. Pharmacol. 39, 533–537.

Bare, L.A., Mansson, E., Yang, D., 1994. Expression of two variants of the human μ-opioid receptor mRNA in SK-N-SH cells and human brain. FEBS Lett. 354, 213–216.

Befort, K., Mattei, M.G., Roeckel, N., Kieffer, B., 1994. Chromosomal localization of the delta opioid receptor gene to human 1p34.3–p36.1 and mouse 4D bands by in situ hybridization. Genomics 20, 143–145.

Belknap, J.K., Crabbe, J.C., 1992. Chromosome mapping of gene loci affecting morphine and amphetamine responses in BXD recombinant inbred mice. Ann. N. Y. Acad. Sci. 654, 311–323.

Belknap, J.K., Mogil, J.S., Helms, M.L., Richards, S.P., O'Toole, L.A., Bergeson, S.E., Buck, K.J., 1995. Localization to chromosome 10 of a locus influencing morphine analgesia in crosses derived from C57BL/6 and DBA/2 strains. Life Sci. 57, PL117–PL124.

Bergen, A.W., Kokoszka, J., Peterson, R., Long, J.C., Virkkunen, M., Linnoila, M., Goldman, D., 1997. μ opioid receptor gene variants: lack of association with alcohol dependence. Mol. Psychiatry 2, 490–494.

Berrettini, W.H., Ferraro, T.N., Alexander, R.C., Buchberg, A.M., Vogel, W.H., 1994. Quantitative trait loci mapping of three loci controlling morphine preference using inbred mouse strains. Nat. Genet. 7, 54–58.

Berrettini, W.H., Hoehe, M.R., Ferraro, T.N., DeMaria, P.A., Gottheil, E., 1997. Human mu opioid receptor gene polymorphisms and vulnerability to substance abuse. Addict. Biol. 2, 303–308.

Bierut, L.J., Dinwiddie, S.H., Begleiter, H., Crowe, R.R., Hesselbrock, V., Nurnberger, J.I. Jr., Porjesz, B., Schuckit, M.A., Reich, T., 1998. Familial transmission of substance dependence: alcohol, marijuana, cocaine, and habitual smoking. Arch. Gen. Psychiatry 55, 982–988.

Bond, C., LaForge, K.S., Mingting, T., Melia, D., Shengwen, Z., Borg, L., Gong, J., Schluger, J., Strong, J.A., Leal, S.M., Tischfield, J.A., Kreek, M.J., Yu, L., 1998. Single-nucleotide polymorphism in the human mu opioid receptor gene alters β-endorphin binding and activity: possible implications for opiate addiction. Proc. Natl. Acad. Sci. U. S. A. 95, 9608–9613.

Cadoret, R.J., O'Gorman, T.W., Troughton, E., Heywood, E., 1985. Alcoholism and antisocial personality. Arch. Gen. Psychiatry 42, 161–167.

Cadoret, R.J., Troughton, E., O'Gorman, T.W., Heywood, E., 1986. An adoption study of genetic and environmental factors in drug abuse. Arch. Gen. Psychiatry 43, 1131–1136.

Cargill, M., Altshuler, D., Ireland, J., Sklar, P., Ardlie, K., Patil, N., Lane, C.R., Lim, E.P., Kalyanaraman, N., Nemesh, J., Ziaugra, L., Friedland, L., Rolfe, A., Warrington, J., Lipshutz, R., Daley, G.Q., Lander, E.S., 1999. Characterization of single-nucleotide polymorphisms in coding regions of human genes. Nat. Genet. 22, 231–238.

Chan, R.J., McBride, A.W., Thomasson, H.R., Ykenney, A., Crabb, D.W., 1994. Allele frequencies of the preproenkephalin A (PENK) gene CA repeat in Asians, African-Americans, and Caucasians: lack of evidence for different allele frequencies in alcoholics. Alcohol Clin. Exp. Res. 18, 533–535.

Chaturvedi, K., Shahrestanifar, M., Howells, R.D., 2000. μ opioid receptor: role for the amino terminus as a determinant of ligand binding affinity. Mol. Brain Res. 76, 64–72.

Chu, J.Y., Huang, W., Kuang, S.Q., Wang, J.M., Xu, J.J., Chu, Z.T., Yang, Z.Q., Lin, K.Q., Li, P., Wu, M., Geng, Z.C., Tan, C.C., Du, R.F., Jin, L., 1998. Genetic relationship of populations in China. Proc. Natl. Acad. Sci. U. S. A. 95, 11763–11768.

Cloninger, R., Bohman, M., Sigvardsson, S., 1981. Inheritance of alcohol abuse: cross-fostering analysis of adopted men. Arch. Gen. Psychiatry 38, 861–868.

Comb, M., Rosen, H., Seeburg, P., Adelman, J., Herbert, E., 1983. Primary structure of the human proenkephalin gene. DNA 2, 213–229.

Comings, D.E., Blake, H., Dietz, G., Gade-Andavolu, R., Legro, R.S., Saucier, G., Johnson, P., Verde, R., MacMurray, J.P., 1999. The proenkephalin gene (PENK) and opioid dependence. NeuroReport 10, 1133–1135.

Connor, M., Christie, M.J., 1999. Opioid receptor signalling mechanisms. Clin. Exp. Pharmacol. Physiol. 26, 493–499.

Culpepper-Morgan, J.A., Kreek, M.J., 1997. Hypothalamic–pituitary adrenal axis hypersensitivity to naloxone in opioid dependence: a case of naloxone-induced withdrawal. Metabolism 46, 130–134.

Echwald, S.M., Sørensen, T.I.A., Andersen, T., Tybjærg-Hansen, A., Clausen, J.O., Pedersen, O., 1999. Mutational analysis of the proopiomelanocortin gene in Caucasians with early onset obesity. Int. J. Obes. 23, 293–298.

Eyre-Walker, A., 1999. Evidence of selection on silent site base composition in mammals: potential implications for the evolution of isochores and junk DNA. Genetics 152, 675–683.

Feder, J., Migone, N., Chang, A.C.Y., Cochet, M., Cohen, S.N., Cann, H., Cavalli-Sforza, L.L., 1983. A DNA polymorphism in close physical linkage with the proopiomelanocortin gene. Am. J. Hum. Genet. 1090–1096.

Feder, J., Gurling, H.M.D., Darby, J., Cavalli-Sforza, L.L., 1985. DNA restriction fragment analysis of the proopiomelanocortin gene in schizophrenia and bipolar disorders. Am. J. Hum. Genet. 37, 286–294.

Franke, P., Nöthen, M.M., Wang, T., Neidt, H., Knapp, M., Lichtermann, D., Weiffenbach, O., Mayer, P., Höllt, V., Propping, P., Maier, W., 1999. Human δ-opioid receptor gene and susceptibility to heroin and alcohol dependence. Neuropsychiatr. Genet. 88, 462–464.

Geijer, T., Telkov, M., Terenius, L., 1995. Characterization of human prodynorphin gene transcripts. Biochem. Biophys. Res. Commun. 215, 881–888.

Geijer, T., Jönason, E., Neiman, J., Gyllander, A., Sedvall, G., Rydberg, U., Terenius, L., 1997. Prodynorphin allelic distribution in Scandinavian chronic alcoholics. Alcohol Clin. Exp. Res. 21, 1333–1336.

Gelernter, J., Kranzler, H., Lacobelle, J., 1998. Population studies of polymorphisms at loci of neuropsychiatric interest (tryptophan hydroxylase (TPH), dopamine transporter protein (SLC6A3), D3 dopamine receptor (DRD3), apolipoprotein E (APOE), (opioid receptor (OPRM1), and ciliary neurotrophic factor (CNTF)). Genomics 52, 289–297.

Gelernter, J., Kranzler, H., Cubells, J., 1999. Genetics of two μ opioid receptor gene (OPRM1) exon 1 polymorphisms: population studies, and allele frequencies in alcohol- and drug-dependent subjects. Mol. Psychiatry 4, 476–483.

Goodwin, D.W., Schulsinger, F., Hermansen, L., Guze, S.B., Winokur, G., 1973. Alcohol problems in adoptees raised apart from alcoholic biological parents. Arch. Gen. Psychiatry 28, 238–243.

Gostout, B., Liu, Q., Sommer, S.S., 1993. "Cryptic" repeating triplets of purines and pyrimidines (cRRY(i)) are frequent and polymorphic: analysis of coding cRRY(i) in the proopiomelanocortin (POMC) and TATA-binding protein (TBP) genes. Am. J. Hum. Genet. 52, 1182–1190.

Grove, W.M., Eckert, E.D., Heston, L., Bouchard, T.J. Jr., Segal, N., Lykken, D.T., 1990. Heritability of substance abuse and antisocial behavior: a study of monozygotic twins reared apart. Biol. Psychiatry 27, 1293–1304.

Halushka, M.K., Fan, J.-B., Bentley, K., Hsie, L., Shen, N., Weder, A., Cooper, R., Lipshutz, R., Chakravarti, A., 1999. Patterns of single-nucleotide polymorphisms in candidate genes for blood-pressure homeostasis. Nat. Genet. 22, 239–247.

Hinney, A., Becker, I., Heibült, O., Nottebom, K., Schmidt, A., Ziegler, A., Mayer, H., Siegfried, W., Blum, W.F., Remschmidt, H., Hebebrand, J., 1998. Systematic mutation screening of the pro-opiomelanocortin gene: identification of several genetic variants including

three different insertions, one nonsense and two missense point mutations in probands of different weight extremes. J. Clin. Endocrinol. Metab. 83, 3737–3741.

Hixson, J.E., Almasy, L., Cole, S., Birnbaum, S., Mitchell, B.D., Mahaney, M.C., Stern, M.P., MacCluer, J.W., Blangero, J., Comuzzie, A.G., 1999. Normal variation in leptin levels is associated with polymorphisms in the proopiomelanocortin gene, *POMC*. J. Clin. Endocrinol. Metab. 84, 3187–3191.

Horikawa, S., Takai, T., Toyosato, M., Takahashi, H., Noda, M., Kakidani, H., Kubo, T., Hirose, T., Inayama, S., Hayashida, H., Miyata, T., Numa, S., 1983. Isolation and structural organization of the human preproenkephalin B gene. Nature 306, 611–614.

Hrubec, Z., Omenn, G.S., 1981. Evidence of genetic predisposition to alcoholic cirrhosis and psychosis: twin concordances for alcoholism and its biological end points by zygosity among male veterans. Alcohol Clin. Exp. Res. 5, 207–215.

Jordanova, A., Kalaydjieva, L., Savov, A., Claustres, M., Schwarz, M., Estivill, X., Angelicheva, D., Haworth, A., Casals, T., Kremensky, I., 1997. SSCP analysis: a blind sensitivity trial. Hum. Mutat. 10, 65–70.

Kaij, L., 1960. Alcoholism in Twins: Studies on the Etiology and Sequels of Abuse of Alcohol. Almqvist & Wiksell, Stockholm.

Kendler, K.S., Prescott, C.A., 1998. Cocaine use, abuse and dependence in a population-based sample of female twins. Br. J. Psychiatry 173, 345–350.

Kendler, K.S., Karkowski, L., Prescott, C.A., 1999. Hallucinogen, opiate, sedative and stimulant use and abuse in a population-based sample of female twins. Acta Psychiatr. Scand. 99, 368–376.

Kidd, K.K., 1993. Associations of disease with genetic markers: déja vu all over again. Neuropsychiatr. Genet. 48, 71–73.

Knapp, R.J., Malatynska, E., Collins, N., Fang, L., Wang, J.Y., Hruby, V.J., Roeske, W.R., Yamamura, H.I., 1995. Molecular biology and pharmacology of the opioid receptors. FASEB J. 9, 516–525.

Koch, T., Kroslak, T., Averbeck, M., Mayer, P., Schröder, H., Raulf, E., Höllt, V., 2000. Allelic variation S268P of the human μ-opioid receptor affects both desensitization and G protein coupling. Mol. Pharmacol. 58, 328–334.

Kranzler, H.P., Gelernter, J., O'Malley, S., Hernandez-Avila, C.A., Kaufman, D., 1998. Association of alcohol or other drug dependence with alleles of the μ opioid receptor gene (OPRM1). Alcohol Clin. Exp. Res. 22, 1359–1362.

Kreek, M.J., 1996a. Opiates, opioids and addiction. Mol. Psychiatry 1, 232–254.

Kreek, M.J., 1996b. Opioid receptors: some perspectives from early studies of their role in normal physiology, stress responsivity, and in specific addictive diseases. Neurochem. Res. 21, 1469–1488.

Kreek, M.J., 2000. Opiates, opioids, SNP's and the addictions: Nathan B. Eddy Memorial Award for lifetime excellence in drug abuse research lecture. In: Harris, L.S. (Ed.), Problems of Drug Dependence, 1999. Proceedings of the 61st Annual Scientific Meeting of the College on Problems of Drug Dependence. National Institute of Drug Abuse Research Monograph Series. Washington, DC: Supt. Of Docs., US Govt. Print Off, DHHS Pub. No. (ADM)00-4737, vol. 180, pp. 3–22.

Kreek, M.J., Koob, G.F., 1998. Drug dependence: stress and dysregulation of brain reward pathways. Drug Alcohol Depend. 51, 23–47.

Kreek, M.J., Wardlaw, S.L., Friedman, J., Schneider, B., Frantz, A.G., 1981. Effects of chronic exogenous opioid administration on levels of one endogenous opioid (beta-endorphin) in man. In: Simon, E., Takagi, H. (Eds.), Advances in Endogenous and Exogenous Opioids. Kondasha, Tokyo, pp. 364–366.

Krude, H., Biebermann, H., Luck, W., Horn, R., Brabant, G., Grüters, A., 1998. Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by *POMC* mutations in humans. Nat. Genet. 19, 155–157.

LaForge, K.S., Kreek, M.J., Uhl, G.R., Sora, I., Yu, L., Befort, K., Filliol, D., Favier, V., Hoehe, M., Kieffer, B.L., Höllt, V., 2000a. Symposium XIII: allelic polymorphisms of human opioid receptors: functional studies: genetic contributions to protection from, or vulnerability to, addictive diseases. In: Harris, F. (Ed.), Problems of Drug Dependence, 1999. Proceedings of the 61st Annual Scientific Meeting of the College on Problems of Drug Dependence. National Institute of Drug Abuse Research Monograph Series, Bethesda, MD: US Department of Health and Human Services, National Institutes of Health. NIH Pub. No. (ADM)00-4737, vol. 180, pp. 47–50.

LaForge, K.S., Shick, V., Spangler, R., Proudnikov, D., Yuferov, V., Lysov, Y., Mirzabekov, A., Kreek, M.J., 2000b. Detection of single nucleotide polymorphisms of the human mu opioid receptor gene by hybridization or single nucleotide extension on custom oligonucleotide gelpad microchips: potential in studies of addiction. Neuropsychiatr. Genet., in press.

Law, P.-Y., Loh, H.H., 1999. Regulation of opioid receptor activities. J. Pharmacol. Exp. Ther. 289, 607–624.

Li, T., Liu, X., Zhu, Z.H., Zhao, J., Hu, X., Sham, P.C., Collier, D.A., 2000. Association analysis of polymorphisms in the μ opioid gene and heroin abuse in Chinese subjects. Addict. Biol. 5, 181–186.

Lin, A.A., Hebert, J.M., Mountain, J.L., Cavalli-Sforza, L.L., 1994. Comparison of 79 DNA polymorphisms tested in Australians, Japanese and Papua New Guineans with those of five other human populations. Gene Geogr. 8, 191–214.

Litt, M., Buroker, N.E., Kondoleon, S., Douglass, J., Liston, D., Sheehy, R., Magenis, R.E., 1988. Chromosomal localization of the human proenkephalin and prodynorphin genes. Am. J. Hum. Genet. 42, 327–334.

Massotte, D., Kieffer, B.L., 1998. A molecular basis for opiate action. Essays Biochem. 33, 65–77.

Matthes, H.W.D., Maldonado, R., Simonin, F., Valverde, O., Slowe, S., Kitchen, I., Befort, K., Dierich, A., Le Meur, M., Dollé, P., Tzavara, E., Hanoune, J., Roques, B.P., Kieffer, B.L., 1996. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the μ opioid receptor gene. Nature 383, 819–823.

Mayer, P., Rochlitz, H., Rauch, E., Rommelspacher, H., Hasse, H.E., Schmidt, S., Höllt, V., 1997. Association between a delta opioid receptor gene polymorphism and heroin dependence in man. NeuroReport 8, 2547–2550.

Merikangas, K.R., Stolar, M., Stevens, D.E., Goulet, J., Presig, M.A., Fenton, B., Zhang, H., O'Malley, S.S., Rounsaville, B.J., 1998. Familial transmission of substance use disorders. Arch. Gen. Psychiatry 55, 973–979.

Mestek, A., Hurley, J.H., Bye, L.S., Campbell, A.D., Chen, Y., Tian, M., Liu, J., Schulman, H., Yu, L., 1995. The human μ opioid receptor: modulation of functional desensitization by calcium/calmodulin-dependent protein kinase and protein kinase C. J. Neurosci. 15, 2396–2406.

Mikesell, M.J., Sobell, J.L., Sommer, S.S., McMurray, C.T., 1996. Identification of a missense mutation and several polymorphisms in the proenkephalin A gene of schizophrenic patients. Neuropsychiatr. Genet. 67, 459–467.

Mikesell, M.J., Barron, Y.D., Nimgaonkar, V.L., Sobell, J.L., Sommer, S.S., McMurray, C.T., 1997. Gly(247) → Asp proenkephalin A mutation is rare in schizophrenia populations. Neuropsychiatr. Genet. 74, 213–215.

Morris, J.C., Savva, D., Lowry, P.J., 1993. Cloning of human proopiomelanocortin cDNA from the pars distalis. J. Endocrinol. 137, P129.

Morris, J.C., Bertram, C.E., Lowry, P.J., Savva, D., 1994. Cryptic trinucleotide repeat polymorphism in the POMC gene. Hum. Mol. Genet. 3, 2080.

Nestler, E.J., Aghajanian, G.K., 1997. Molecular and cellular basis of addiction. Science 278, 58–63.

Owerbach, D., Rutter, W.J., Roberts, J.L., Whitfeld, P., Shine, J., Seeburg, P.H., Shows, T.B., 1981. The proopiocortin (adrenocorticotropin/β-lipotropin) gene is located on chromosome 2 in humans. Somatic Cell Genet., 359–369.

Partanen, J., Bruun, K., Markkanen, T., 1966. Inheritance of Drinking Behavior: A Study on Intelligence, Personality, and Use of Alcohol of Adult Twins. The Finnish Foundation for Alcohol Studies, Helsinki.

Pickens, R.W., Dace, S.S., McGue, M., Lykken, D.T., Heston, L.L., Clayton, P.J., 1991. Heterogeneity in the inheritance of alcoholism: a study of male and female twins. Arch. Gen. Psychiatry 48, 19–28.

Poulsen, L., Brøsen, K., Arendt-Nielsen, L., Gram, L.F., Elbæk, K., Sindrup, S.H., 1996. Codeine and morphine in extensive and poor metabolizers of sparteine: pharmacokinetics, analgesic effect and side effects. Eur. J. Clin. Pharmacol. 51, 289–295.

Roberts, A.J., McDonald, J.S., Heyser, C.J., Kieffer, B.L., Matthes, H.W.D., Koob, G.F., Gold, L.H., 2000. μ-opioid receptor knockout mice do not self-administer alcohol. J. Pharmacol. Exp. Ther. 293, 1002–1008.

SAMHSA, National Household Survey on Drug Abuse Series: H-12, 2000. Summary of findings from the 1999 National Household Survey on Drug Abuse, DHHS Publication Number (SMA)00-3466, Rockville, MD.

Sander, T., Gscheidel, N., Wendel, B., Samochowiec, J., Smolka, M., Rommelspacher, H., Schmidt, L.G., Hoehe, M.R., 1998. Human μ-opioid receptor variation and alcohol dependence. Alcohol Clin. Exp. Res. 22, 2108–2110.

Sander, T., Berlin, W., Gscheidel, N., Wendel, B., Janz, D., Hoehe, M.R., 2000. Genetic variation of the human μ-opioid receptor and susceptibility to idiopathic absence epilepsy. Epilepsy Res. 39, 57–61.

Satoh, H., Mori, S., 1997. Subregional assignment of the proopiomelanocortin gene (POMC) to human chromosome band 2p23.3 by fluorescence in situ hybridization. Cytogenet. Cell Genet. 76, 221–222.

Shippenberg, T.S., Elmer, G.I., 1998. The neurobiology of opiate reinforcement. Crit. Rev. Neurobiol. 12, 267–303.

Simonin, F., Gavériaux-Ruff, C., Befort, K., Matthes, H., Lannes, B., Micheletti, G., Mattei, M.G., Charron, G., Bloch, B., Kieffer, B., 1995. κ-opioid receptor in humans: cDNA and genomic cloning, chromosomal assignment, functional expression, pharmacology, and expression pattern in the central nervous system. Proc. Natl. Acad. Sci. U. S. A. 92, 7006–7010.

Smolka, M., Sander, T., Schmidt, L., Samochowiec, J., Rommelspacher, H., Gscheidel, N., Wendel, B., Hoehe, M.R., 1999. μ-opioid receptor variants and dopaminergic sensitivity in alcohol withdrawal. Psychoneuroendocrinology 24, 629–638.

Standifer, K.M., Pasternak, G.W., 1997. G proteins and opioid receptor-mediated signalling. Cell. Signalling 9, 237–248.

Stocker, S., 1998. New NIDA initiative focuses on vulnerability to drug addiction. NIDA Notes, National Institute on Drug Abuse, National Institutes of Health, Bethesda, MD 13, Publication 98-3478, 1–4.

Strong, J.A., Dalvi, A., Samaha, F.J., Gong, J., Xu, K., Yue, X., Yu, L., 1999. Mu opioid receptor polymorphism and L-DOPA induced dyskinesia in Parkinson disease. Soc. Neurosci. 394.

Takahashi, H., Teranishi, Y., Nakanishi, S., Numa, S., 1981. Isolation and structural organization of the human corticotropin-β-lipotropin precursor gene. FEBS Lett. 135, 97–102.

Takahashi, H., Hakamata, Y., Watanabe, Y., Kikuno, R., Miyata, T., Numa, S., 1983. Complete nucleotide sequence of the human corticotropin-β-lipotropin precursor gene. Nucleic Acids Res. 11, 6847–6858.

Town, T., Abdullah, L., Crawford, F., Schinka, J., Ordorica, P.I., Francis, E., Hughes, P., Duara, R., Mullan, M., 1999. Association of a functional μ-opioid receptor allele (+118A) with alcohol dependency. Neuropsychiatr. Genet. 88, 458–461.

Tsuang, M.T., Lyons, M.J., Eisen, S.A., Goldberg, J., True, W., Lin, N., Meyer, J.M., Toomey, R., Faraone, S.V., Eaves, L., 1996. Genetic influences on DSM-III-R drug abuse and dependence: a study of 3,372 twin pairs. Neuropsychiatr. Genet. 67, 473–477.

Tsuang, M.T., Lyons, M.J., Meyer, J.M., Doyle, T., Eisen, S.A., Goldberg, J., True, W., Lin, N., Toomey, R., Eaves, L., 1998. Co-occurrence of abuse of different drugs in men: the role of drug specific and shared vulnerabilities. Arch. Gen. Psychiatry 55, 967–972.

Tyndale, R.F., Droll, K.P., Sellers, E.M., 1997. Genetically deficient CYP2D6 metabolism provides protection against oral opiate dependence. Pharmacogenetics 7, 375–379.

Uhl, G.R., Sora, I., Wang, Z., 1999. The μ opiate receptor as a candidate gene for pain: polymorphisms, variations in expression, nociception, and opiate responses. Proc. Natl. Acad. Sci. U. S. A. 96, 7752–7755.

Wang, J.B., Johnson, P.S., Persico, A.M., Hawkins, A.L., Griffin, C.A., Uhl, G.R., 1994. Human μ opiate receptor: cDNA and genomic clones, pharmacologic characterization and chromosomal assignment. FEBS Lett. 338, 217–222.

Weber, J.L., May, P.E., 1990. Dinucleotide repeat polymorphism at the PENK locus; Dinucleotide repeat polymorphism at the D2S72 locus. Nucleic Acids Res. 18, 2200.

Wendel, B., Hoehe, M.R., 1998. The human μ opioid receptor gene: 5′ regulatory and intronic sequences. J. Mol. Med. 70, 525–532.

Yasuda, K., Espinosa, R. III, Takeda, J., LeBeau, M.M., Bell, G.I., 1994. Localization of the kappa opioid receptor gene to human chromosome band 8q11.2. Genomics 19, 596–597.

Zabel, B.U., Naylor, S.L., Sakaguchi, A.Y., Bell, G.I., Shows, T.B., 1983. High-resolution chromosomal localization of human genes for amylase, proopiomelanocortin, somatostatin, and a DNA fragment (D3S1) by in situ hybridization. Proc. Natl. Acad. Sci. U. S. A. 80, 6932–6936.

Zimprich, A., Kraus, J., Wöltje, M., Mayer, P., Rauch, E., Höllt, V., 2000. An allelic variation in the human prodynorphin gene promoter alters stimulus-induced expression. J. Neurochem. 74, 472–477.