HHS Public Access

Author manuscript

J Hum Genet. Author manuscript; available in PMC 2011 August 01.

Published in final edited form as:

J Hum Genet. 2011 February ; 56(2): 147–155. doi:10.1038/jhg.2010.150.

Haplotype block structure of the genomic region of the mu opioid receptor gene (*OPRM1*)

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Abstract

The opioid system is involved in the action of opiate drugs, opioid addiction, pain experience and analgesia. Individual differences in opioid effect may be attributed in part to genetic variations. Long-range *cis* regulatory elements and intronic variants are potential sources of functional diversity. Recently, we have detected association of two intronic *OPRM1* variants with heroin addiction in European Americans. In the current study, we analyzed the genetic variations in the *OPRM1* 100 kb 5′ flanking region and intron 1 in the HapMap Caucasian population. Four major linkage disequilibrium (LD) blocks were identified, consisting of 28, 22, 15 and 42 SNPs, respectively. The locations of these blocks are (−100 – −90), (−90 – −67), (−20 - −1) and (+1 – +44) kb, respectively. The two intronic variants, indicated in our recent study, are part of a distinct haplogroup that include SNPs from intron 1, and the proximal 5′ region. The 118G (rs1799971) allele is part of a different haplogroup that includes several variants in the distal 5′ region that may have a regulatory potential. These findings were corroborated by genotyping eight SNPs in a sample of European Americans and suggest an extended *OPRM1* locus with potential new regulatory regions.

Keywords

LD blocks; distal 5′ region; opioid addiction; genetic variations; haplotype

Introduction

The G protein-coupled mu opioid receptor (encoded by the *OPRM1* gene) affects signal transduction pathways that mediate the effects of opioids and plays an important role in

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Conflict of Interest statement. None declared

Supplementary information is available at *The* Journal of Human Genetics website

opioid reward, tolerance, analgesia and dependence. The vulnerability to develop opioid addiction is partially inherited and individual differences in opioid effects may be attributed to genetic variations. Understanding the effects of this variability is of clinical importance.

The non-synonymous variant rs1799971 (118A>G, Asn40Asp) was shown to remove a potential *N*-glycosylation site in the extracellular domain, to be more potent in beta-endorphin binding and receptor activity, and to reduce receptor signaling efficacy.1 Healthy subjects with the 118G allele showed an increased basal level of cortisol and greater cortisol responses to opioid receptor blockade with naloxone in a population-specific manner.2-4 Several studies reported positive association of the 118G variant with opioid dependence and other substance dependencies, in diverse populations,5-7 while other studies did not detect association.8, 9 The clinical relevance of this polymorphism for opioid analgesia and opioid adverse effects is still debatable.10-12 The 118G allele frequencies varied between populations, with high frequency in Asian populations (0.35-0.48), moderate frequency in European populations (0.1-0.17), and low frequency in African populations (<0.04).13

Another potential source of functional diversity is splice variants. At least half of the genome is expressed in alternatively spliced isoforms14 and gene functions may also be modified by SNPs in alternatively spliced exons. A number of *OPRM1* alternatively spliced variants have been reported in rodents and humans.15-20 The receptors encoded by two human splice variants that retain different parts of intron 1 were shown to form heterodimers with the wild type protein.19 An alternatively spliced exon with an alternative promoter, in the 5′ upstream region (~28-30 kb), was identified in rodents and humans, along with cell-specific splice variants.15, 19, 21-23 A SNP in intron 1 showed an effect on the *OPRM1* gene expression *in vitro* and was associated with pain sensitivity.20

The proximal promoter region does not necessarily contain all elements required for tissue-specific gene expression, and regulatory elements can be located great distances from the gene they regulate.14, 24 Only a few potential *OPRM1* regulatory variants have been functionally characterized. SNP −554G>A decreased OPRM1 activity in a neuroblastoma cell line, and SNP −1320A>G showed increased OPRM1 activity.22, 25 Association studies of potential regulatory *OPRM1* variants with substance dependence were limited to the proximal regulatory region. An association with substance dependence was shown with SNPs −1793T>A, −1699insT, and −2044C>A.26, 27

In a recent association study of heroin addiction we have detected association of two variants in intron 1 (rs510769 and rs3778151) in European Americans.9 Of note, the “addiction-focused” array28 used in this study does not include *OPRM1* SNPs upstream of exon 1. These results are supported by two studies in which intron 1 SNPs showed association with drug dependence in European Americans,29 or with positive response to heroin after first use in Chinese.30 We did not find a similar association in an African American cohort.31 A 10K genome-wide association study of a subset of the same cohort from our laboratory identified association of a SNP rs1074287, located 11.6 kb upstream of exon 1, with heroin addiction.32

Our hypothesis is that there may be additional non-coding variants, possibly distant from *OPRM1*, that contribute to the individual phenotype (e.g. response to opiates, vulnerability to develop opioid addiction, and perception of pain). In addition, a combination of linked variants could contribute to the phenotypic difference in a different way than a single polymorphism.

Toward this goal, we performed an analysis of genetic variations and haplogroups in the *OPRM1* 100 kb 5′ flanking region, exon 1, and the 50 kb intron 1, and searched for regions in high LD with the 118G and/or the intron 1 variants. Genotype data were obtained from the International HapMap Project and from tag SNPs in a sample of European Americans.

Materials and Methods

Comparative genomic analysis

Comparative genomic analysis was obtained from the UCSC Genome Browser (http://genome.ucsc.edu/) and is based on the Vertebrate Multiz Alignment & PhastCons Conservation. Regulatory Potential data were based on the ESPERR Regulatory Potential analysis that is computed from alignments of human, chimpanzee, macaque, mouse, rat, dog, and cow. 33

HapMap data

HapMap genotype and phased haplotype data were obtained from the genome browser of The International HapMap Project Phase 2 (http://www.hapmap.org/). The sample populations used were CEU (Utah residents with Northern and Western European ancestry from the CEPH collection), YRI (Yoruba in Ibadan, Nigeria) and the combined JPN/CHB (Japanese in Tokyo and Han Chinese in Beijing). Only the founders in these sample populations were included. The phased haplotype data was generated using a maximum likelihood algorithm. The chromosomal region was set to position 154,300–154,450 kb (build 37.1).

Subjects

An exploratory representative group of 103 subjects was selected, based on their 118A>G genotype, from our European American cohort, which was collected for genetic studies with heroin addiction as described.9 To reduce the potential effect of population stratification, only subjects with a European ancestry proportion of >0.7 based on AIMs analysis were included (see below). The group can be divided as follows: 1) ”118G”/”118G” (n=5); 2) “REF”/”118G” (n=52); 3); “IVS1”/”118G” (n=17); and 4) ”REF”/“REF” (n=29). Since the 118G allele was not found to be associated with heroin addiction in this cohort, both cases (former heroin addicts in methadone maintenance treatment) and controls were included, except for subgroup 3 that included only control subjects. Subjects were recruited at the Rockefeller University Hospital, Weill Medical College of Cornell University, and the Dr. Miriam and Sheldon G. Adelson Clinic for Drug Abuse Treatment and Research in Las Vegas, NV. The Institutional Review Boards of the Rockefeller University Hospital and Cornell University approved the study for the three institutions. All subjects signed informed consent for genetic studies.

Ancestry informative markers (AIMs)

One hundred seventy-four AIMs with adequate quality were employed to calculate the proportion of European ancestry using STRUCTURE34 with the CEPH diversity panel of 1051 individuals, as a reference.9, 28, 35

Linkage disequilibrium (LD) structure, haplotypes and multi-locus genotype patterns (MLGPs)

The pattern of pair-wise LD between the three SNPs (#5, 6 and 8) in the original sample9 was measured by D' and r^2 metrics. Haplotypes were reconstructed using the accelerated expectation-maximization algorithm implemented in Haploview version 4.2.36 Multi-locus genotype patterns were generated manually.

SNP selection and genotyping

Three SNPs (rs1799971(#5), rs510769 (#6) and rs3778151 (#8)) were genotyped using the Illumina custom array.9 Additional five tag SNPs were selected for genotyping from the 100 kb 5′ flanking region (blocks 1: rs1551808 (#1), rs7758009 (#2), and rs7760028 (3#), and block 4: rs1074287 (#4)), and intron 1 (block 4: rs3778146 (#7)) (Table 1, Figure 1, and Supplement Table 1). The selection was based on information from HapMap, chromosomal location and frequency (MAF> 0.1 in CEU).

DNA was extracted from blood and genotypes were determined by TaqMan® technology. The TaqMan® Pre-Designed SNP Genotyping Assay catalog numbers (Applied Biosystems (ABI), Foster City, CA) are listed in Supplement Table 1. PCR was performed in duplicate on a GeneAmp® PCR 9700 using TaqMan® universal PCR master mix with AmpErase uracil-*N*-glycosylate (ABI) according to the manufacturer's protocol. Briefly, TaqMan® assay mix (40x) and universal PCR master mix (2x) were mixed and the volume was adjusted to 4 μl in a 384-well optical reaction plate; 10 ng of genomic DNA (1 μl) were added. PCR amplification lasted for 2 min at 50°C; 10 min at 95°C followed by 40 cycles of 15 sec at 92°C and 1 min at 60°C. Genotype analysis was performed on an ABI Prism® 7900 sequence detection system using SDS 2.1 software (ABI).

For verification of the TaqMan® assay, PCR amplification was performed using Platinum PCR Supermix (Invitrogen, Carlsbad, CA). Primers were designed using software Primer3.37 PCR amplification consisted of 2 min at 94°C; eight 'touch-down' cycles of 30 sec at 94°C, 30 sec at 63-56°C and 30 sec at 72°C; 30 cycles of 30 sec at 94°C, 30 sec at 56°C and 30 sec at 72°C; and a final step of 7 min at 72°C. PCR products were purified and sequenced on an ABI Prism 3700® capillary sequencer (ABI). Electropherograms were scored using the Sequencer 4.5 software (Gene Codes Corporation, Ann Arbor, MI).

Results

The genomic organization of the *OPRM1* gene region is shown in Figure 1a. *OPRM1* is mapped to chromosome 6q25.2 at position 154.4–154.7 Mb (NCBI build 37.1) and is flanked at the 5′ end by three non-functional pseudogenes. The next gene upstream to *OPRM1*, the regulator of G-protein signaling 17 gene (*RGS17*), is located at a 1 mega base

distance at position 153.4. At the 3′ end, *OPRM1* is overlapped by the phosphoinositide-binding protein PIP3-E gene (*IPCEF1)*, in the opposite orientation.

In this study, we focused on the region spanning the ~100 kb 5′ flanking region, exon 1, and the 50 kb intron 1. Sequence comparison of this region between humans, non-human primates and rodents is shown on Figure 1b. Analysis of the LD matrix of the selected region in the HapMap CEU sample revealed that 108 SNPs are located in four major blocks of high LD (Figure 2a, Table 1). The first block spans ~10 kb with 28 SNPs, the second block spans ~23 kb with 22 SNPs, the third block spans ~20 kb with 15 SNPs and the fourth block spans intron 1 (~44 kb) with 42 SNPs. SNPs analyzed in related association studies (except for 118A>G that were studied in many studies) are indicated by their references (also see Discussion).

The HapMap CEU haplotype data revealed four major haplogroups (Figure 2b): 1) the "reference" group ("REF", ~36%) with no variants (including the reference 118A allele and the reference intron 1 sequence); 2) the block 2 group (~22%); 3) the 118G group (~15%) with additional variants in block 1; and 4) the intron 1 variants of block 4 ("INT1", ~11%). The minor haplogroups are: block 3 (4%) and 12% of the haplotypes that could not be assigned to one of the major haplogroups (grouped as "Others" in Figure 2b). Of the 59 HapMap CEU subjects, ten (17%) were homozygous for the "REF" haplogroup, two (3%) were homozygous for the 118G haplogroup, and five (8%) were homozygous for the block 2 haplogroup. No individual was homozygous for the "INT1" haplogroup and one was heterozygous for the "118G"/"INT1" groups. All the rest were heterozygotes with different allelic combinations.

The two SNPs (rs510769 (#6), rs3778151 (#8)) identified in our recent study to be associated with heroin addiction9 are part of the 42-SNP block 4 that spans intron 1. The average MAF of the SNPs of block 4 is 0.15 in European Americans. Interestingly, four SNPs (rs9478498, rs9478499, rs1074287 (#4) and rs6936615), located at the −20 kb 5′ region (block 3 region) are in high LD with the SNPs of block 4. Four SNPs (rs7748401, rs10457090, rs3778152 and rs563649) located at intron 1 are not part of block 4 and are in high LD with the SNPs of block 3. Haplogroup "INT1" includes the reference 118A allele (Table 1). Seven SNPs (rs9384167, rs12174208, rs11966947, rs7760028 (#3), rs12527423, rs17084868, and rs17084870) are in high LD with SNPs in block 1, but are physically located in or adjacent to the region of block 2 (Table 1, Figure 2a). The region that spans block 1 (−100 kb - −90 kb) shows several regions with a regulatory potential (Supplement Figure 1).

We searched for similar haplogroups in the other HapMap populations (the combined JPT/CHB and YRI). The "INT1" haplogroup that includes the intron 1 variants of block 4 accounts for ~4% in these populations. In contrast, the "118G" haplogroup has a very different pattern. In concordance with the 118G allele frequency, the 118G haplogroup that includes the 5′ variants of block 1, is very frequent in the Asian population and can be divided into two subgroups, the major one (28%) includes the variants in block 1, similar to European Americans, and the second (14%) has other combinations (data not shown). The 118G allele was not found in the African sample but a similar haplogroup with block 1

variants (without the 118G variant allele) was represented by 7.5% of the haplotypes. Interestingly, there was a significant representation (~30%) of the block 3 haplogroup, with variants at the proximal 5′ region, in the African sample, in contrast to the low frequency in the Asian and Caucasian samples.

Three major haplogroups were identified in our previous study of European Americans (350 cases and 184 controls) based on *OPRM1* exon 1 SNP rs1799971 (118A>G, #5), and intron 1 SNPs rs510769 (#6) and rs3778151 (#8).9 The major haplogroups identified are: 1) "REF" (65%, 56%); 2) "118G" (13%, 11%); and 3) "INT1" (13%, 20%) in controls and cases, respectively. The higher frequency of the "REF" group may be explained by the fact that the haplogroup of "block 2" cannot be distinguished from the reference group by the three SNPs genotyped. These results demonstrate the higher frequency of the "INT1" haplogroup in the cases that was the basis for the association of this haplogroup with heroin addiction. The allele frequencies in controls are compatible with the HapMap data from Caucasians.

To further explore the finding that the 118G haplogroup includes variant alleles in the distant 5′ region, a representative group of 103 subjects with and without the 118G allele was selected from the original cohort. This exploratory sample does not represent the general population and was selected to enable unambiguous phase determination. To reduce population admixture, only subjects with a European ancestry proportion of >0.7, based on AIMs analysis, were included. The exploratory representative sample is composed of the following subgroups (Table 2): 1) "118G"/"118G" (homozygotes for the 118G allele, n=5); 2) "REF"/"118G" (heterozygotes for 118G allele that are non-carriers of the IVS 1 variants, n=52); 3) "IVS1"/"118G" (double heterozygotes for 118A>G and the two IVS1 SNPs, n=17); and 4) "REF"/"REF" (represents of the reference genotype group, n=29, all controls).

All subjects were genotyped for an additional five SNPs (rs1551808 (#1), rs7758009 (#2), rs7760028 (#3), rs1074287 (#4), and rs3778146 (#7)) (Table 1, Figure 1). The SNPs were selected from the 100 kb 5′ flanking region, and intron 1 based on LD information from HapMap (CEU blocks 1 and 4) and frequency in CEU (MAF> 0.1). As is shown in Table 2, the five subjects with the "118G"/"118G" pattern (subgroup 1) carry two copies of the three variant alleles (#1-3) of the SNPs from the 5′ region (C-G-C, block 1), two copies of the reference A allele of the 5′ SNP rs1074287 (#4), and two copies for the reference allele of the three SNPs from intron 1 (C-T-T, #6-8). The majority of the heterozygotes for the 118G allele ("REF"/"118G", subgroup 2) also carry at least one variant allele of the SNPs #1-3 (TC-AG-TC), and none of them carries the variant allele of SNP rs1074287 (#4) or the three IVS1 SNPs (#6-8). The subjects in subgroup "IVS1"/"118G" (subgroup 4) all carry at least one variant allele of SNPs #1-4 (TC-AG-TC-AG). Although the phase in these subjects cannot be determined unambiguously, the genotypes most probably reflect a combination of two haplogroups: 1) SNP #4/ IVS1 SNPs #6-8 and 2) 118G/ 5′ SNPs #1-3, based on the other subgroups in which the phase can be determined with certainty. This subgroup represents a small group of the original cohort (5% in controls and 8% in cases). The majority of the "REF"/REF" subgroup (subgroup 3) does not carry a variants allele of any of the genotyped SNPs. This exploratory data corroborates the HapMap data that the 118G

haplogroup includes variant alleles of SNPs in the distant 5′ region and is distinct from the "INT1" haplogroup.

Discussion

Genetic diseases can be caused by disruption of the regulatory elements and the identification of these regulatory elements is an important and challenging problem. Tissue-specific and developmental-specific expression requires sophisticated expression profiles that may involve multiple enhancer elements. Long-range *cis* elements and intronic variants may play a role in this regulation. For example, an evolutionarily conserved enhancer was identified 86 kb upstream of the peptidylarginine deiminases gene (*PADI3*) that previous studies limited to the proximal promoter failed to explain its expression pattern.38

Recently, an alternatively spliced exon with a specific promoter was identified in humans, ~28 kb upstream of exon 1,15 suggesting an extended *OPRM1* gene. It is also of note that except for three pseudogenes, there is no gene located at one mega base 5′ to *OPRM1*.

In our hypothesis-driven association study of heroin addiction, two *OPRM1* intron 1 variants (#6, #8) were found at a significantly higher frequency in subjects with heroin addiction than in healthy controls, suggesting an association with heroin addiction.9 A 10K genome-wide association study from our laboratory that used a subset of the sample population, used in the hypothesis-driven study, indicated association of a SNP rs1074287 (#4) in the 5′ flanking region (−11.6 kb from exon 1) with heroin addiction.32 Analysis of the HapMap CEU LD data in this study revealed a unique haplogroup ("INT1") of 42 SNPs that spans intron 1 and also includes at least four variants in the 20 kb 5′ flanking region, including rs1074287. This data indicate that the finding of the two studies are compatible and refer to the same "IVS1" haplogroup. Based on this data, the intron 1 SNPs that show association with heroin addiction may be markers in high LD with regulatory variants in the proximal 5′ region. It is also possible that the SNPs in the 5′ flanking region are markers for functional SNPs in intron 1.

Several lines of evidence suggest an additional functionality to intron 1 beyond the basic splicing of the constitutive exons. Several alternatively spliced exons were described in this intron.19, 20 The finding of a high LD between SNPs spanning the entirety of intron 1 and a few SNPs in the proximal 5′ flanking region, in different populations, is intriguing. This haplogroup is represented at a relatively low frequency (4%) in Asian and African populations but at a much higher frequency (15%) in the European population, suggesting the possibility of an ancient haplotype with a specific selective advantage in Europeans. It is also possible that this population's difference occurs by random as was recently suggested in a genome-wide study of genes with highly divergent allele frequencies between populations. 39

A potential functional SNP in intron 1 (rs563649) was recently indicated in association with pain perception.20 The HapMap LD data indicate that although this SNP not part of the "INT1" haplogroup, it is in high LD with SNPs of haplogroup 3 that includes variants in the proximal 5′ region (block 3). Notably, this haplogroup is rare in European Americans, but

frequent in the African sample. It is possible that this SNP is a marker of regulatory variant/s in the proximal 5′ region.

Many studies provided evidence for the importance of the *OPRM1* 118A>G polymorphism in addiction, pain, HPA activation, and treatment response.[e.g., 2-9] These studies have shown mixed results regarding the association between the 118G variant and opioid dependence, opioid analgesia and opioid adverse effects.40 Most studies have used single SNP analyses and were generally limited to the coding region or to the proximal upstream region. Several studies employed haplotype analysis but were also limited to specific regions. Hoehe *et al.* performed a comprehensive haplotype analysis of *OPRM1* but the study did not include SNPs from intron 1 and was limited to the proximal 5′ region (−2.4 kb). 26 Lue *et al.* studied five variants from the proximal 5′ flanking region (−2 kb) and two SNPs from exon 1. 27 Zhang *et al*. examined 13 SNPs spanning the coding sequence including several SNPs from intron 1, but none in the 5′ flanking region. 29, 30 Xuei *et al.* genotyped 18 SNPs including one in the proximal 5′ region (−6 kb). 41 A recent study used a haplotype-based approach to predict response to naltrexone treatment based on 10 *OPRM1* tag SNPs but was limited to the proximal 5′ region (−11 kb).42 Haplotype-based analysis is generally considered more powerful than single-marker analysis and may help to determine if a specific polymorphism occurs on a common genetic background.

The mixed single SNP association results for *OPRM1* SNP 118A>G may be explained by different haplotype patterns. For example, the finding that about a third of the chromosomes with the 118G allele in the HapMap Asian populations do not show high LD with block 1 SNPs suggests the existence of a second 118G haplogroup that is specific to this population. It is feasible that single SNP association studies of the 118A>G SNP in this population may result in mixed results. The data obtained in this study indicate that, in European Americans, the 118G variant allele is positioned within a specific haplogroup and is in high LD with several distant variants that may possibly have a regulatory effect. It remains to be shown whether these variants are correlated with *OPRM1* expression levels. . The variant 118G allele was not found in the African HapMap population suggesting that this is a relatively new variant that arose after the out-of-Africa migration of modern humans. The high prevalence of this allele in the Asian population and the lesser prevalence in European Americans may be explained by genetic drift and/or selective advantage. Interestingly, a similar haplogroup (block 1), without the 118G allele, was found in the African HapMap sample suggesting that the change to 118G may have occurred on this ancestral haplotype.

In summary, the identification of LD blocks and haplogroups in the *OPRM1* intron 1 and the 5′ flanking region may be of importance to the field of opioid response and addiction. This study suggests an extended *OPRM1* locus and possible new regulatory regions. The haplotype architecture described in this study may provide direction for future genetic studies. These findings will require confirmation by functional expression studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

The authors would like to thank Pei-Hong Shen and David Goldman from the Laboratory of Neurogenetics (NIAAA) for the AIMs analysis, to the clinical staff including Elizabeth Ducat, Brenda Ray, Dorothy Melia, and Lisa Borg, for patient recruitment and ascertainment, and toVadim Yuferov, Ann Ho, David Nielsen, Matthew Randesi and Susan Russo for their contributions.

This work was supported by the National Institutes of Health [P60-DA 05130 to M.J.K.]

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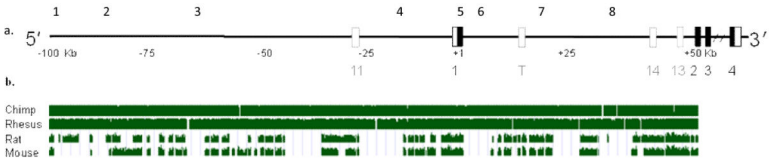

Figure 1.

a. Schematic representation of the *OPRM1* gene region and the location of the 8 SNPs genotyped: rs1551808 (1), rs7758009 (2), rs7760028 (3), rs1074287 (4), rs1799971 (118A>G, 5), rs510769 (6), rs3778146 (7), and rs3778151 (8). Black boxes indicate exons, open boxes indicate untranslated regions (UTR) and dotted boxes indicate alternatively spliced exons.

b. A customized view from the UCSC genome browser that covers 100 kb of the 5′ region, exon 1 and 50 Kb of intron 1, showing pair-wise alignment of each of four species with the human genome. The tracks from top to bottom show the positions of segments aligned with the indicated comparison species. Green bars indicate conserved regions.

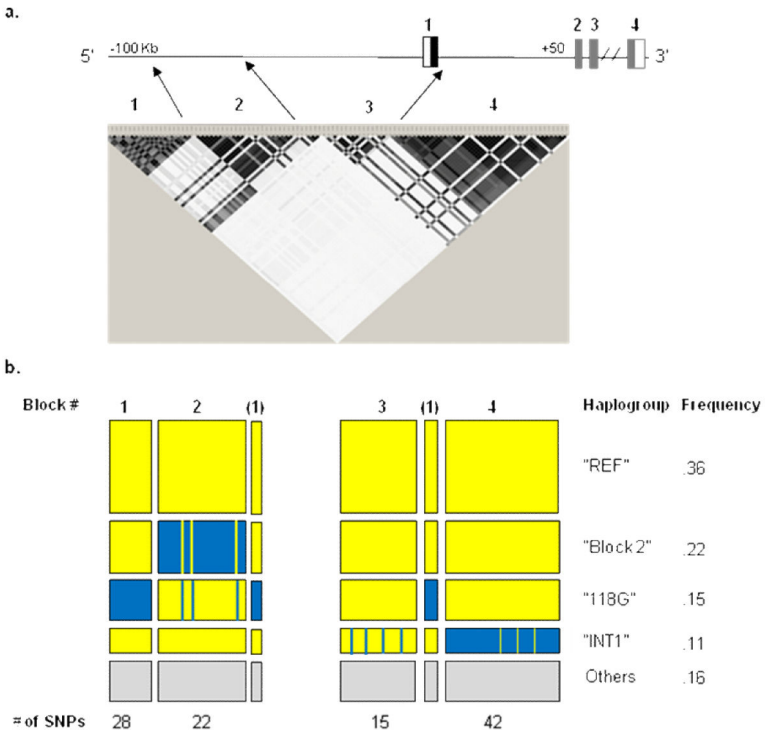

Figure 2.

a. Plot of pair-wise linkage disequilibrium (LD) of the 150 kb *OPRM1* genomic region, in the HapMap CEU sample. The plot is not drawn to scale and the arrows show the correct position of the SNPs. The color scheme indicates the magnitude of r^2. Black blocks indicate $r^2 = 1$, and white blocks indicate $r^2 = 0$. **b**. The pattern and frequency of the major haplogroups in this population. The height of the boxes is proportional to the frequency in the population. Blue boxes indicate variant alleles, yellow boxes indicate reference alleles and gray boxes indicate all the other haplogroups.

J Hum Genet. Author manuscript; available in PMC 2011 August 01.

Table 1

LD haplotype block distributionof *OPRM1* SNPs in the HapMap CEU population

#	LD Block	Position	SNPs				MAF	References[a]
			Haplo-group 1	Haplo-group 2	Haplo-group 3	Haplo-group 4		
1	Block 1 (5′ region)	154301883	rs3829284				0.21	
2		154302640	***rs155180***				0.28	
3		154303286	rs116638				0.39	
4		154304373	rs282095				0.39	
5		154304587	rs1885629				0.25	
6		154304631	rs1885628				0.25	
7		154304972	rs282096				0.39	
8		154305678	rs204426				0.39	
9		154307218	rs605249				0.36	
10		154307715	rs1708472				0.25	
11		154308258	rs7745695				0.35	
12		154308291	rs9397672				0.25	
13		154308367	rs7741685				0.34	
14		154308402	rs9397673				0.25	
15		154308430	rs7745892				0.38	
16		154308693	rs9397674				0.25	
17		154308840	rs2169411				0.22	
18		154309097	rs1033817				0.35	
19		154309787	rs6557329				0.25	
20		154310237	rs1033816				0.25	
21		154311203	***rs775800***				0.22	
22	Block 2 (5′ region)	154312749		rs472615			0.25	
23		154312816		rs626174			0.25	
24		154 312831		rs626150			0.25	
25		154313470		rs612692			0.25	
26		154313492		rs612665			0.25	

#	LD Block	Position	SNPs				MAF	References[a]
			Haplo-group 1	Haplo-group 2	Haplo-group 3	Haplo-group 4		
27		154313761		rs611357			0.25	
28		154314057		rs610040			0.25	
29		154314311		rs598832			0.16	
30		154314407		rs525479			0.26	
31		154314435		rs5005184			0.25	
32		154315619		rs582542			0.25	
33		154315643		rs493335			0.25	
34		154316767	rs9384167				0.23	
35		154319896		rs613515			0.25	
36		154320006		rs613030			0.25	
37		154320077	rs1217420				0.22	
38		154320301		rs601509			0.28	
39		154323894		rs546 536			0.28	
40		154324902		rs647303			0.24	
41		154325561		rs488966			0.28	
42		154326611		rs282108			0.24	
43		154329645		rs282103			0.25	
44		154329725		rs6900805			0.24	
45		154330276	rs1196694				0.23	
46		154335481		rs484565			0.20	
47		15433 6766	***rs776002***				0.23	
48		154354079	rs1252742				0.22	
49		154363627	rs1708486				0.22	
50		154364271	rs1708487				0.22	
51	Block 3 (5′ region)	154382310			rs12214130		0.05	
52		154383069				rs9478498	0.23	
53		154383251			rs12216066		0.06	
54		154383485			rs12216210		0.06	
55		154386982				rs9478499	0.14	

#	LD Block	Position	SNPs				MAF	References[a]
			Haplo-group 1	Haplo-group 2	Haplo-group 3	Haplo-group 4		
56		154388025			rs12192681		0.06	
57		154389936			rs7776131		0.06	
58		154390181			rs7751510		0.06	
59		154 390298			rs7776341		0.06	20
60		154390502				***rs1074287***	0.23	20,32,
61		154391394			rs6910065		0.06	
62		154396793				rs6936615	0.15	
63		154399679			rs12210856		0.06	
64		154400626			rs12205732		0.05	26
65		154402201			rs6912029		0.05	26
66	Exon 1	154402490	***rs179997***					
67	Block 4 (Intron 1)	154403712				***rs510769***	0.23	9,30,4
68		154403947				rs9322445	0.15	
69		154405080				rs477292	0.19	
70		154405995				rs557748	0.18	
71		154406133				rs634479	0.18	
72		1544 08773				rs3778145	0.14	
73		154409772				rs668394	0.18	
74		154409860				rs514980	0.18	
75		154410240				rs511435	0.18	29,41
76		154410479				rs509544	0.18	
77		154414130				rs607759	0.18	
78		154414385				rs499796	0.19	
79		154416785				rs524731	0.18	20,29,4
80		154417218				rs520321	0.18	
81		154419618				***rs3778146***	0.14	
82		154420432				rs3778147	0.14	
83		154420845				rs3823010	0.14	29
84		154421627				rs9285542	0.14	

#	LD Block	Position	SNPs				MAF	References[a]
			Haplo-group 1	Haplo-group 2	Haplo-group 3	Haplo-group 4		
85		154421843			rs7748401		0.05	
86		154422705				rs3778148	0.14	
87		154423832				rs3778149	0.14	
88		154424060				rs7773995	0.14	
89		154424166				rs7772959	0.14	
90		154424751				rs9479754	0.14	
91		154425351				rs3778150	0.14	
92		154426224				rs9478501	0.14	
93		154429999				rs6927269	0.14	
94		154432766			rs10457090		0.05	
95		154433062				rs1461773	0.14	
96		154434368				rs9478503	0.15	
97		154434951				rs1381376	0.15	20,29,30
98		154435373				***rs3778151***	0.17	30
99		154435524			rs3778152		0.04	
100		154435577				rs3778153	0.15	
101		154436852				rs9478504	0.15	
102		154438148				rs17209711	0.15	
103		154438165				rs17275521	0.15	
104		154445511				rs3778155	0.14	
105		154447394				rs3778157	0.15	
106		154447423				rs3778158	0.15	
107		154447744				rs3798678	0.15	
108		154449660			rs563649		0.08	20,41

SNPs in bold italics were genotyped forthis study.

[a]References indicating SNPs that were analyzed in other studies.

Table 2

Multi-locus genotype patterns (MLGPs) in a representative sample of the 118A>GGenotype subgroups

	rs1551808	rs7758009	rs7760028	rs1074287	rs1799971	rs510769	rs3778146	rs3778151	
	1	2	3	4	5	6	7	8	Phenotype
1. “118G”/”118G“									
1	CC	GG	CC	AA	GG	CC	TT	TT	control
2	CC	GG	CC	AA	GG	CC	TT	TT	control
3	CC	GG	CC	AA	GG	CC	TT	TT	control
4	CC	GG	CC	AA	GG	CC	TT	TT	case
5	CC	GG	CC	AA	GG	CC	TT	TT	case
2. “REF”/”118G”									
6	TC	AG	TC	AA	AG	CC	TT	TT	case
7	TC	AG	TC	AA	AG	CC	TT	TT	case
8	TC	AG	TC	AA	AG	CC	TT	TT	case
9	TC	AG	TC	AA	AG	CC	TT	TT	case
10	TC	AG	TC	AA	AG	CC	TT	TT	case
11	TC	AG	TC	AA	AG	CC	TT	TT	case
12	TC	AG	TC	AA	AG	CC	TT	TT	case
13	TC	AG	TC	AA	AG	CC	TT	TT	case
14	TC	AG	TC	AA	AG	CC	TT	TT	case
15	TC	AG	TC	AA	AG	CC	TT	TT	case
16	TC	AG	TC	AA	AG	CC	TT	TT	case
17	TC	AG	TC	AA	AG	CC	TT	TT	case
18	TC	AG	TC	AA	AG	CC	TT	TT	case
19	TC	AG	TC	AA	AG	CC	TT	TT	case
20	TC	AG	TC	AA	AG	CC	TT	TT	case
21	TC	AG	TC	AA	AG	CC	TT	TT	case
22	TC	AG	TC	AA	AG	CC	TT	TT	case
23	TC	AG	TC	AA	AG	CC	TT	TT	case
24	TC	AG	TC	AA	AG	CC	TT	TT	case
25	TC	AG	TC	AA	AG	CC	TT	TT	case

	rs1551808	rs7758009	rs7760028	rs1074287	rs1799971	rs510769	rs3778146	rs3778151	
	1	2	3	4	5	6	7	8	Phenotype
26	TC	AG	TC	AA	AG	CC	TT	TT	case
27	TC	AG	TC	AA	AG	CC	TT	TT	case
28	TC	AG	TC	AA	AG	CC	TT	TT	case
29	TC	AG	TC	AA	AG	CC	TT	TT	case
30	TC	AG	TC	AA	AG	CC	TT	TT	case
31	TC	AG	TC	AA	AG	CC	TT	TT	case
32	TC	AG	TC	AA	AG	CC	TT	TT	case
33	TC	AG	TC	AA	AG	CC	TT	TT	control
34	TC	AG	TC	AA	AG	CC	TT	TT	control
35	TC	AG	TC	AA	AG	CC	TT	TT	control
36	TC	AG	TC	AA	AG	CC	TT	TT	control
37	TC	AG	TC	AA	AG	CC	TT	TT	control
38	TC	AG	TC	AA	AG	CC	TT	TT	control
39	TC	AG	TC	AA	AG	CC	TT	TT	control
40	TC	AG	TC	AA	AG	CC	TT	TT	control
41	TC	AG	TC	AA	AG	CC	TT	TT	control
42	TC	AG	TC	AA	AG	CC	TT	TT	control
43	TC	AG	TC	AA	AG	CC	TT	TT	control
44	TC	AG	TC	AA	AG	CC	TT	TT	control
45	TC	AG	TC	AA	AG	CC	TT	TT	control
46	TC	AG	TC	AA	AG	CC	TT	TT	control
47	TC	AG	TC	AA	AG	CC	TT	TT	control
48	TC	AG	TC	AA	AG	CC	TT	TT	control
49	TC	AG	TC	AA	AG	CC	TT	TT	control
50	TC	AG	TC	AA	AG	CC	TT	TT	control
51	TC	AG	TC	AA	AG	CC	TT	TT	control
52	CC	AA	TT	AA	AG	CC	TT	TT	control
53	CC	AA	TT	AA	AG	CC	TT	TT	control
54	CC	AA	TT	AA	AG	CC	TT	TT	case
55	CC	AA	TT	AA	AG	CC	TT	TT	case

	rs1551808	rs7758009	rs7760028	rs1074287	rs1799971	rs510769	rs3778146	rs3778151	
	1	2	3	4	5	6	7	8	Phenotype
56	CC	GG	CC	AA	AG	CC	TT	TT	case
57	CC	GG	CC	AA	AG	CC	TT	TT	case
3. “IVS1”/”118G”									
58	TC	AG	TC	AG	AG	CT	TC	TC	control
59	TC	AG	TC	AG	AG	CT	TC	TC	control
60	TC	AG	TC	AG	AG	CT	TC	TC	case
61	TC	AG	TC	AG	AG	CT	TC	TC	case
62	TC	AG	TC	AG	AG	CT	TC	TC	case
63	TC	AG	TC	AG	AG	CT	TC	TC	case
64	TC	AG	TC	AG	AG	CT	TC	TC	case
65	TC	AG	TC	AG	AG	CT	TC	TC	case
66	TC	AG	TC	AG	AG	CT	TC	TC	case
67	TC	AG	TC	AG	AG	CT	TC	TC	case
68	TC	AG	TC	AG	AG	CT	TC	TC	case
69	TC	AG	TC	AG	AG	CT	TC	TC	case
70	TC	AG	TC	AG	AG	CT	TC	TC	case
71	CC	AG	TC	AG	AG	CT	TC	TC	case
72	CC	AG	TC	AG	AG	CT	TC	TC	case
73	TC	GG	TC	AG	AG	CT	TC	TC	case
74	CC	GG	CC	AG	AG	CT	TC	TC	case
4. “REF“/”REF”									
75	CC	AA	TT	AA	AA	CC	TT	TT	control
76	CC	AA	TT	AA	AA	CC	TT	TT	control
77	CC	AA	TT	AA	AA	CC	TT	TT	control
78	CC	AA	TT	AA	AA	CC	TT	TT	control
79	CC	AA	TT	AA	AA	CC	TT	TT	control
80	CC	AA	TT	AA	AA	CC	TT	TT	control
81	TT	AA	TT	AA	AA	CC	TT	TT	control
82	TT	AA	TT	AA	AA	CC	TT	TT	control
83	TT	AA	TT	AA	AA	CC	TT	TT	control

	rs1551808	rs7758009	rs7760028	rs1074287	rs1799971	rs510769	rs3778146	rs3778151	
	1	2	3	4	5	6	7	8	Phenotype
84	TT	AA	TT	AA	AA	CC	TT	TT	control
85	TT	AA	TT	AA	AA	CC	TT	TT	control
86	TT	AA	TT	AA	AA	CC	TT	TT	control
87	TT	AA	TT	AA	AA	CC	TT	TT	control
88	TT	AA	TT	AA	AA	CC	TT	TT	control
89	TT	AA	TT	AA	AA	CC	TT	TT	control
90	TT	AA	TT	AA	AA	CC	TT	TT	control
91	TT	AA	TT	AA	AA	CC	TT	TT	control
92	TT	AA	TT	AA	AA	CC	TT	TT	control
93	TT	AA	TT	AA	AA	CC	TT	TT	control
94	TT	AA	TT	AA	AA	CC	TT	TT	control
95	TT	AA	TT	AA	AA	CC	TT	TT	control
96	TT	AA	TT	AA	AA	CC	TT	TT	control
97	TT	AA	TT	AA	AA	CC	TT	TT	control
98	TT	AA	TT	AA	AA	CC	TT	TT	control
99	TT	AA	TT	AA	AA	CC	TT	TT	control
100	TT	AA	TT	AA	AA	CC	TT	TT	control
101	TT	AA	TT	AA	AA	CC	TT	TT	control
102	TT	AA	TT	AA	AA	CC	TT	TT	control
103	TT	AA	TT	AA	AA	CC	TT	TT	control