Published in final edited form as:

Drug Alcohol Depend. 2011 August 1; 117(1): 62–65. doi:10.1016/j.drugalcdep.2010.12.026.

No evidence of association between 118A>G *OPRM1* polymorphism and heroin dependence in a large Bulgarian case-control sample

Momchil A. Nikolov^{1,4}, Olga Beltcheva¹, Antoaneta Galabova², Anna Ljubenova³, Elena Jankova³, Galin Gergov², Atanas A. Russev³, Michael T. Lynskey⁴, Elliot C. Nelson⁴, Eleonora Nesheva², Dorita Krasteva², Philip Lazarov², Vanio I. Mitev¹, Ivo M. Kremensky⁶, Radka P. Kaneva¹, and Alexandre A. Todorov⁴

¹Molecular Medicine Center and Department of Medical Chemistry and Biochemistry, Medical University – Sofia, Sofia 1431, Bulgaria

²Bulgarian Addictions Institute, Sofia 1619, Bulgaria

³Initiative for Health Foundation, Sofia 1680, Bulgaria

⁴Washington University in Saint Louis, School of Medicine, Department of Psychiatry – St. Louis MO 63110, USA

⁶National Genetic Laboratory, University Hospital of Obstetrics and Gynecology “Majchin dom”, Medical University – Sofia, Sofia 1431, Bulgaria

Abstract

The μ -opioid receptor is the primary site of action of most opioids. The 118A>G (rs1799971) polymorphism in exon 1 of the μ -opioid receptor gene (*OPRM1*) leads to an Asn40Asp amino acid change that affects a putative N-glycosylation site. It has been widely investigated for association with alcohol and drug dependence and pain sensitivity, with mixed results. The aim of the current study was to examine whether this polymorphism was associated with heroin dependence in a large Bulgarian cohort of 1842 active users and 1451 population controls. SNP genotyping was done using Real-Time PCR TaqMan technology. Association analyses were conducted, separately for Roma and non-Roma participants. Our results suggest that there is no direct effect of 118A>G genotype on the risk for heroin dependence among active heroin users.

© 2011 Elsevier Ireland Ltd. All rights reserved.

Corresponding author Alexandre A. Todorov, PHD, Department of Psychiatry, Washington University School of Medicine, 660 South Euclid Avenue, Saint Louis MO 63110, Tel: (314) 747-6774, Fax: (314) 747-6777, todorov@wustl.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Contributors

Momchil A. Nikolov conducted the analyses and wrote the paper. Participant recruitment and face-to-face interviews were led by Dorita Krasteva, Eleonora Nesheva, Elena Jankova, Anna Ljubenova, Antoaneta Galabova, Galin Gergov and Atanas A. Russev. Olga Beltcheva was responsible for biosample management. Elliot C. Nelson, Michael T. Lynskey, Ivo M. Kremensky, Philip Lazarov and Radka P. Kaneva are study co-investigators. Alexandre A. Todorov developed study design, supervised data management and statistical analysis. All authors contributed to and approved the final manuscript.

Conflict of interest

There are no conflicts of interest.

Keywords

Heroin dependence; *OPRM1*; association; 118A>G

1. Introduction

OPRM1 (OMIM: 600018) is a natural candidate gene for studies of opioid dependence (OD) as it encodes the μ -opioid receptor, the primary target of opioids. A common polymorphism in exon 1 of *OPRM1* (rs1799971 or 118A>G) results in an Asn40Asp amino-acid change. Initial reports suggested the 118G variant could increase the receptor's affinity for β -endorphin, its endogenous ligand (Bond et al., 1998). Though this was not confirmed in subsequent work (Befort et al., 2001; Beyer et al., 2004), other studies indicate that the 118G allele might still alter *OPRM1* function, either by lowering *OPRM1* expression (Beyer et al., 2004) or through a deleterious effect on mRNA and protein yield (Drakenberg et al., 2006; Zhang et al., 2005).

The possible functional relevance of this polymorphism prompted a number of studies, across several ethnicities, to examine its effect on the risk of developing opioid dependence. Results have been mixed, with some indication for association with opioid dependence in Indians belonging to the Kayastha and Brahmin castes of the Bengali-Hindu ethnic background, Indian, and Hong Kong Chinese samples (Deb et al., 2010; Tan et al., 2003; Kapur et al., 2007; Szeto et al., 2001) and negative results in American-Indian, African-American, European-American, European, Chinese, Malays and Han Chinese samples (Arias et al., 2006; Crowley et al., 2003; Franke et al., 2001; Glatt et al., 2007; Shi et al., 2002; Tan et al., 2003; Zhang et al., 2007). Haplotypes from two SNPs (rs510769, rs3778151) show nominally significant association with opioid dependence, but do not include the 118A>G polymorphism although they are in linkage disequilibrium with it (Levrán et al., 2008). In a meta-analysis of 22 studies including 8000 subjects divided by ethnicity and substance dependence type, the 118A>G polymorphism did not appear to affect the risk for substance dependence in general (Arias et al., 2006). Another meta-analysis, focusing strictly on opioid dependence, also failed to find evidence for association with the 118A>G polymorphism, in 21 case-control studies representing 1742 opioid dependent cases and 2585 control subjects from European, African, Asian and Native American ancestry (Glatt et al., 2007).

However, until a recent Australian study (E.C. Nelson, personal correspondence), most previous work used relatively low sample sizes with the number of cases ranging from 77 (Tan et al., 2003) to 486 (Xu et al., 2004). Previous analyses may thus have been statistically underpowered. The present study re-examines this issue as part of an ongoing study of opioid dependence in Bulgaria, using samples from ethnic Bulgarians and Romas, two previously unstudied populations.

2. Methods

2.1. Participants

The sample consists of 1842 active heroin users (1516 ethnic Bulgarian and 326 Roma), the majority of whom have not received regular care for their drug problem (such as methadone maintenance). Participants are aged 18 years and older, have used heroin daily or nearly daily for a minimum of one year prior to assessment and met DSM-IV criteria for a lifetime diagnosis of heroin dependence. The 1451 unrelated population-representative control samples include 1178 ethnic Bulgarians and 273 Romas. Recruitment (via snowball sampling) is still underway, with the targeted sample size of 2,500 cases and 2,500 controls

to be reached in 2011. Face-to-face semi-structured psychiatric interviews were done by trained members of the research team. The number of control samples was balanced to cases on gender and ethnicity. Within gender and ethnicity, the control sample is population representative, being selected from the biobank of the PKU newborn screening program at the National Genetic Laboratory in Sofia (250,000 samples, > 85% of all live births since 1998). Given the low prevalence of heroin dependence in the population (under 1%), the use of population controls is not expected to result in a loss of statistical power. The study was conducted with approvals from the IRBs of Washington University in Saint Louis and the Medical University in Sofia. All participants gave informed consent.

2.2. Procedures

DNA was extracted from venous blood samples from each heroin dependent subject, and from 10mm punches (PKU Guthrie cards) for controls, using chemagic Magnetic Separation Module I (chemagen AG) according to manufacturer's protocol. A Biomek FX^P robot was used for DNA aliquoting in 384 well plates and TaqMan assays for genotyping the 118A>G polymorphism on a 7900HT Fast Real-Time PCR System (both from Applied Biosystems). SDS v2.2.2 analysis software tool was used for base-calling and visualization of the genotype data. For quality control purposes, four samples from each plate were re-genotyped on another plates (no errors were detected). The call rate for the five case and the four control 384 plates was over 95 %. The data were analyzed using PLINK (Purcell et al., 2007) and SAS® software. Statistical power analyses were done using Quanto (Gauderman et al., 2006).

3. Results

Of the 1842 cases, 1528 (83%) were males and 314 (17%) females. Of the 1451 controls, 1142 (79%) were males and 309 (21%) females. The mean participant age was 30 (SD 5.7). Table 1 summarizes allelic and genotypic frequencies for 118A>G. Within ethnicity, we found no significant deviations from Hardy-Weinberg equilibrium in either cases or controls. The G allele is more common in Romas than in ethnic Bulgarians (20.2% versus 13.8%, $\chi^2(1) = 11.2$, $p = 0.0009$). The association analyses were thus carried out independently for the two ethnic groups to avoid possible stratification problems. Within ethnicity, we find no significant difference in allelic or genotypic frequencies between heroin dependent cases and controls. Among Roma women, there was a weak trend toward a higher frequency of the G allele in heroin dependent participants (45.1% compared to 31.8%, $\chi^2(1) = 2.16$, $p = 0.14$). However, the relevant samples sizes ($N = 51$ and 66 , respectively) are too limited for definite conclusions.

The frequency of the G allele in Bulgarian controls did not differ from that reported in other Europeans samples (13.8% compared to 17% for HapMap-CEU), and was very close to those observed in an Australian study (E.C. Nelson, personal correspondence) for general population controls (15.5% on $N=1494$) and controls from socioeconomically disadvantaged neighbourhoods (12.5% on $N=531$). There are no reference frequencies for this polymorphism in Bulgarian Romas, a population that is believed to have Indian origins (Gresham et al., 2001). In Romas, the observed frequency of the G allele (20.2%) was significantly different to that reported for Indians from the Kayastha and Brahmin castes of the Bengali-Hindu ethnic background sample (28%, $\chi^2(1) = 35.68$, $p < 0.0001$) (Deb et al., 2010), and from those reported in Tan et al. (47%, $\chi^2(1) = 72.6$, $p < 0.0001$) for an Indian sample (Tan et al., 2003).

This study was reasonably powered to replicate previous findings. Under the dominant mode of inheritance, a G allele frequency of ~13% consistent with our data, and conservatively assuming population prevalence of 0.5%, we estimate that the current study had 80% power

to detect a genetic relative risk of ~1.45 and higher in the ethnic Bulgarian male sample alone at the $\alpha = 0.001$ significance level; and 80% power for a genetic relative risk of ~1.32 at $\alpha = 0.05$. These are lower than the smallest estimated relative risk of ~1.6 from previous positive association results. The results are virtually identical assuming co-dominance. Power is low to detect a recessive mode of inheritance with 17.3% frequency of 118G allele.

4. Discussion

This is the first study to examine whether the 118A>G polymorphism in *OPRM1* is associated with an increased risk for opioid dependence in ethnic Bulgarian and Roma active heroin users. Previous association studies in several populations worldwide have produced mixed results in that regard. Our results indicate that 118A>G is not directly associated with heroin dependence in our sample, consistent with other studies in African-American, Caucasian, Han Chinese and European American populations (Crowley et al., 2003; Franke et al., 2001; Zhang et al., 2007; Zhang et al., 2006a). It is unlikely that other polymorphisms in exon 1 of *OPRM1* played a role. In other work, we sequenced this exon in a separate sample of 101 heroin dependent cases and 101 controls, all ethnic Bulgarians (ABI310, Applied Biosystems). The frequency of the 118G allele was very close to what we ultimately observed in the main study (14% compared to 13.8%). Other known SNPs, including rs1799972, rs1799973, rs1042753, rs9282817 and rs9282819 were virtually monomorphic. The rare ins/del GGC at codon 63 (rs9282818) was observed in only one subject.

Women were well represented in this sample (17.3% and 15.6% of ethnic Bulgarian and Roma cases, respectively; close to the proportion observed in populations of heroin users). The slightly higher frequency of the 118G allele in heroin dependent Roma women raises the possibility of sex-specific differences, as suggested, for example, by studies of the effect of this polymorphism on reduced sensitivity to pain (Fillingim et al., 2005) and alcohol-dependence (Kim, 2009). Moreover, in a mouse model, Mague et al. found sex-specific effects of the 118A>G polymorphism on drug mediated behaviors, such as reductions in the rewarding properties of morphine and the aversive components of naloxone-precipitated morphine withdrawal (Mague et al., 2009).

The accumulating findings linking polymorphisms and haplotypes in *OPRM1*, as well as epigenetic changes with opioid, alcohol and nicotine dependence and treatment responses do warrant further investigations to elucidate the contribution of genetic variations to *OPRM1* expression, the risk for opioid dependence and failure to abstain (Bart et al., 2005; Hernandez-Avila et al., 2003; Levran et al., 2008; Oroszi et al., 2009; Shabalina et al., 2009; Wand et al., 2002; Yuferov et al., 2010; Zhang et al., 2007; Zhang et al., 2006a; Zhang et al., 2006b). It is possible, for example, that the action of the 118A>G polymorphism is not in increasing the risk to develop opioid dependence but rather its severity. This is suggested by a study in a Han Chinese sample, where no association was found between 118A>G and drug dependence, but where haplotypes involving the 118A>G and the IVS2 +31 G/A variant were associated with higher tolerance in heroin dependent individuals (Shi et al., 2002). However, this study, the large scale Australian study (E.C. Nelson, personal correspondence), and recent meta-analyses indicate that there is no direct association between this polymorphism alone and heroin dependence.

Acknowledgments

The technical contribution of Romyana Dodova, Mina Angelova, Darina Kachakova, Gergana Stancheva, and Emilia Boyadjieva from the Molecular Medicine Center, Medical University - Sofia is gratefully acknowledged.

Funding source

Supported by grant R01 DA018823 from the National Institute on Drug Abuse to Alexandre Todorov and T32 training grant GM081739 from National Institute of General Medical Sciences to Randall Larsen

References

- Arias A, Feinn R, Kranzler HR. Association of an Asn40Asp (A118G) polymorphism in the mu-opioid receptor gene with substance dependence: a meta-analysis. *Drug Alcohol Depend.* 2006; 83:262–268. [PubMed: 16387451]
- Bart G, Kreek MJ, Ott J, LaForge KS, Proudnikov D, Pollak L, Heilig M. Increased attributable risk related to a functional mu-opioid receptor gene polymorphism in association with alcohol dependence in central Sweden. *Neuropsychopharmacology.* 2005; 30:417–422. [PubMed: 15525999]
- Befort K, Filliol D, Decaillot FM, Gaveriaux-Ruff C, Hoehe MR, Kieffer BL. A single nucleotide polymorphic mutation in the human mu-opioid receptor severely impairs receptor signaling. *J. Biol. Chem.* 2001; 276:3130–3137. [PubMed: 11067846]
- Beyer A, Koch T, Schroder H, Schulz S, Holtt V. Effect of the A118G polymorphism on binding affinity, potency and agonist-mediated endocytosis, desensitization, and resensitization of the human mu-opioid receptor. *J. Neurochem.* 2004; 89:553–560. [PubMed: 15086512]
- Bond C, LaForge KS, Tian M, Melia D, Zhang S, Borg L, Gong J, Schluger J, Strong JA, Leal SM, Tischfield JA, Kreek MJ, Yu L. Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. *Proc. Natl. Acad. Sci. U S A.* 1998; 95:9608–9613. [PubMed: 9689128]
- Crowley JJ, Oslin DW, Patkar AA, Gottheil E, DeMaria PA Jr, O'Brien CP, Berrettini WH, Grice DE. A genetic association study of the mu opioid receptor and severe opioid dependence. *Psychiatr. Genet.* 2003; 13:169–173. [PubMed: 12960749]
- Deb I, Chakraborty J, Gangopadhyay PK, Choudhury SR, Das S. Single-nucleotide polymorphism (A118G) in exon 1 of OPRM1 gene causes alteration in downstream signaling by mu-opioid receptor and may contribute to the genetic risk for addiction. *J. Neurochem.* 2010; 112:486–496. [PubMed: 19891732]
- Drakenberg K, Nikoshkov A, Horvath MC, Fagergren P, Gharibyan A, Saarelainen K, Rahman S, Nylander I, Bakalkin G, Rajs J, Keller E, Hurd YL. Mu opioid receptor A118G polymorphism in association with striatal opioid neuropeptide gene expression in heroin abusers. *Proc. Natl. Acad. Sci. U S A.* 2006; 103:7883–7888. [PubMed: 16682632]
- Fillingim RB, Kaplan L, Staud R, Ness TJ, Glover TL, Campbell CM, Mogil JS, Wallace MR. The A118G single nucleotide polymorphism of the mu-opioid receptor gene (OPRM1) is associated with pressure pain sensitivity in humans. *J. Pain.* 2005; 6:159–167. [PubMed: 15772909]
- Franke P, Wang T, Nothen MM, Knapp M, Neidt H, Albrecht S, Jahnes E, Propping P, Maier W. Nonreplication of association between mu-opioid-receptor gene (OPRM1) A118G polymorphism and substance dependence. *Am. J. Med. Genet.* 2001; 105:114–119. [PubMed: 11424981]
- Gauderman, WJ.; Morrison, JM. Quanto1.1: A computer program for power and sample size calculations for genetic-epidemiology studies. 2006. Available: <http://hydra.usc.edu/gxe>.
- Glatt SJ, Bousman C, Wang RS, Murthy KK, Rana BK, Lasky-Su JA, Zhu SC, Zhang R, Li J, Zhang B, Lyons MJ, Faraone SV, Tsuang MT. Evaluation of OPRM1 variants in heroin dependence by family-based association testing and meta-analysis. *Drug Alcohol Depend.* 2007; 90:159–165. [PubMed: 17416470]
- Gresham D, Morar B, Underhill PA, Passarino G, Lin AA, Wise C, Angelicheva D, Calafell F, Oefner PJ, Shen P, Tournev I, de Pablo R, Kucinkas V, Perez-Lezaun A, Marushiakova E, Popov V, Kalaydjieva L. Origins and divergence of the Roma (gypsies). *Am. J. Hum Genet.* 2001; 69:1314–1331. [PubMed: 11704928]
- Hernandez-Avila CA, Wand G, Luo X, Gelernter J, Kranzler HR. Association between the cortisol response to opioid blockade and the Asn40Asp polymorphism at the mu-opioid receptor locus (OPRM1). *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 2003; 118B:60–65. [PubMed: 12627468]
- Kapur S, Sharad S, Singh RA, Gupta AK. A118g polymorphism in mu opioid receptor gene (oprm1): association with opiate addiction in subjects of Indian origin. *J. Integr. Neurosci.* 2007; 6:511–522. [PubMed: 18181266]

- Kim SG. Gender differences in the genetic risk for alcohol dependence--the results of a pharmacogenetic study in Korean alcoholics. *Nihon Arukoru Yakubutsu Igakkai Zasshi*. 2009; 44:680–685. [PubMed: 20077761]
- Levrán O, Londono D, O'Hara K, Nielsen DA, Peles E, Rotrosen J, Casadonte P, Linzy S, Randesi M, Ott J, Adelson M, Kreek MJ. Genetic susceptibility to heroin addiction: a candidate gene association study. *Genes Brain Behav*. 2008; 7:720–729. [PubMed: 18518925]
- Mague SD, Isiegas C, Huang P, Liu-Chen LY, Lerman C, Blendy JA. Mouse model of OPRM1 (A118G) polymorphism has sex-specific effects on drug-mediated behavior. *Proc. Natl. Acad. Sci. USA*. 2009; 106:10847–10852. [PubMed: 19528658]
- Oroszi G, Anton RF, O'Malley S, Swift R, Pettinati H, Couper D, Yuan Q, Goldman D. OPRM1 Asn40Asp predicts response to naltrexone treatment: a haplotype-based approach. *Alcohol Clin. Exp. Res*. 2009; 33:383–393. [PubMed: 19053977]
- Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet*. 2007; 81:559–575. [PubMed: 17701901]
- Shabalina SA, Zaykin DV, Gris P, Ogurtsov AY, Gauthier J, Shibata K, Tchivileva IE, Belfer I, Mishra B, Kiselycznyk C, Wallace MR, Staud R, Spiridonov NA, Max MB, Goldman D, Fillingim RB, Maixner W, Diatchenko L. Expansion of the human mu-opioid receptor gene architecture: novel functional variants. *Hum. Mol. Genet*. 2009; 18:1037–1051. [PubMed: 19103668]
- Shi J, Hui L, Xu Y, Wang F, Huang W, Hu G. Sequence variations in the mu-opioid receptor gene (OPRM1) associated with human addiction to heroin. *Hum. Mutat*. 2002; 19:459–460. [PubMed: 11933204]
- Szeto CY, Tang NL, Lee DT, Stadlin A. Association between mu opioid receptor gene polymorphisms and Chinese heroin addicts. *Neuroreport*. 2001; 12:1103–1106. [PubMed: 11338173]
- Tan EC, Tan CH, Karupathivan U, Yap EP. Mu opioid receptor gene polymorphisms and heroin dependence in Asian populations. *Neuroreport*. 2003; 14:569–572. [PubMed: 12657887]
- Wand GS, McCaul M, Yang X, Reynolds J, Gotjen D, Lee S, Ali A. The mu-opioid receptor gene polymorphism (A118G) alters HPA axis activation induced by opioid receptor blockade. *Neuropsychopharmacology*. 2002; 26:106–114. [PubMed: 11751037]
- Xu K, Lichtermann D, Lipsky RH, Franke P, Liu X, Hu Y, Cao L, Schwab SG, Wildenauer DB, Bau CH, Ferro E, Astor W, Finch T, Terry J, Taubman J, Maier W, Goldman D. Association of specific haplotypes of D2 dopamine receptor gene with vulnerability to heroin dependence in 2 distinct populations. *Arch. Gen. Psychiatry*. 2004; 61:597–606. [PubMed: 15184239]
- Yuferov V, Levran O, Proudnikov D, Nielsen DA, Kreek MJ. Search for genetic markers and functional variants involved in the development of opiate and cocaine addiction and treatment. *Ann. N.Y. Acad. Sci*. 2010; 1187:184–207. [PubMed: 20201854]
- Zhang D, Shao C, Shao M, Yan P, Wang Y, Liu Y, Liu W, Lin T, Xie Y, Zhao Y, Lu D, Li Y, Jin L. Effect of mu-opioid receptor gene polymorphisms on heroin-induced subjective responses in a Chinese population. *Biol. Psychiatry*. 2007; 61:1244–1251. [PubMed: 17157823]
- Zhang H, Luo X, Kranzler HR, Lappalainen J, Yang BZ, Krupitsky E, Zvartau E, Gelernter J. Association between two mu-opioid receptor gene (OPRM1) haplotype blocks and drug or alcohol dependence. *Hum. Mol. Genet*. 2006a; 15:807–819. [PubMed: 16476706]
- Zhang L, Kendler KS, Chen X. The mu-opioid receptor gene and smoking initiation and nicotine dependence. *Behav. Brain Funct*. 2006b; 2:28. [PubMed: 16887046]
- Zhang Y, Wang D, Johnson AD, Papp AC, Sadee W. Allelic expression imbalance of human mu opioid receptor (OPRM1) caused by variant A118G. *J. Biol. Chem*. 2005; 280:32618–32624. [PubMed: 16046395]

Table 1
Allelic and genotypic frequencies of the *OPRM1* 118A>G polymorphism, by opioid dependence status, ethnicity and gender.

	Males		Females		Bulgarian males		Roma males		Bulgarian females		Roma females	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Genotype	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
G/G	35 (2.9)	34 (3.8)	9 (3.3)	9 (3.5)	24 (1.9)	23 (2.4)	11 (4.0)	11 (5.3)	7 (2.7)	6 (2.5)	2 (3.9)	3 (4.5)
A/G	387 (26.8)	276 (26.8)	69 (29.7)	74 (25.1)	308 (24.6)	212 (22.7)	79 (29.0)	64 (30.9)	48 (18.2)	56 (23.0)	21 (41.2)	18 (27.3)
A/A	1106 (70.2)	832 (69.3)	236 (67.0)	226 (71.3)	921 (73.5)	700 (74.9)	185 (67.0)	132 (63.8)	208 (79.1)	181 (74.5)	28 (54.9)	45 (68.2)
Total	1528	1142	314	309	1253	935	275	207	263	243	51	66
allele												
G	16.3%	17.3%	18.1%	16.1%	14.2%	13.8%	18.4%	20.8%	11.8%	14.0%	24.5%	18.2%
A	83.7%	82.7%	81.8%	83.9%	85.8%	86.2%	81.6%	79.2%	88.2%	86.0%	75.5%	81.8%