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Association of the functional A118G polymorphism of *OPRM1* in diabetic patients with foot ulcer pain

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Abstract

Background: Diabetic foot ulcer (DFU) patients may experience moderate or severe pain. A single-nucleotide polymorphism, at nucleotide 118 for opioid receptor mu 1 (*OPRM1*), has been reported to alter the opioid effects to relieve acute or chronic pain. The purpose of this study was to elucidate the correlation between nucleotide 118 variants and foot ulcer pain in DFU patients. **Methods:** Sixty-five DFU patients with Grade 2–5 Wagner–Meggitt classification were enrolled. The occurrence of pain in activities was categorized into five grades. Patients were allocated either into the painless DFU group, with a visual analog scale (VAS) pain score ≦3, or into the painful DFU group, with a VAS pain score ≧4 and Grades 3–5 of occurrence of pain in daily activities. DNA was extracted from blood samples of analyzed patients. Using the polymerase chain reaction–single-strand conformation polymorphism analysis and DNA sequencing of nucleotide 118, we identified the genotype distribution and allelic frequencies in DFU patients. The sequences of the forward and the reverse primer are designed as follows: 5′-TAATACGACTCACTATAGGG-3′ and 5′-ACGCACACGATGGAGTAGAG-3′, respectively. **Results:** Fifteen patients were classified into the painful DFU group and 50 patients were classified into the painless DFU group. The amplified DNA fragments showed 26 homozygous (AA), 34 heterozygous (AG), and 5 mutant homozygous (GG) genotypes, with overall A and G allelic frequencies of 66.2% and 33.8%, respectively. The painful DFU group included 10 AA subjects, 4 AG subjects, and 1 GG subject, while the painless DFU group had 16 AA, 30 AG, and 4 GG subjects (P=.038). **Conclusion:** The A118G polymorphism of mu-opioid receptor may be closely associated with DFU pain in 34 out of 50 patients in the painless group and in 5 out of 15 patients in the painful group. This indicates that the nucleotide 118 variant patients may suffer less DFU pain.

Keywords: Diabetes; Single-nucleotide polymorphism; Mu-opioid receptor; *OPRM1*; Foot ulcer pain

1. Introduction

Diabetic foot ulcer (DFU) is one of the major complications of diabetes mellitus (DM), and the lifetime risk of a diabetic patient developing a foot ulcer may be as high as 25% (Singh, Armstrong, & Lipsky, 2005). The most common pathway to diabetic foot ulceration has been

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identified as peripheral sensory impairment either with acute mechanical or thermal trauma or with repetitively or continuously applied mechanical stress (Mayfield, Reiber, Sanders, Janisse, & Pogach, 2003; Ulbrecht, Cavanagh, & Caputo, 2004). Additionally, diabetic patients with peripheral arterial disease more easily suffer ischemic foot ulceration than those without the disease (Dolan et al., 2002; Gregg et al., 2004). Patients with DFU may experience moderate to severe pain (Freedman, Cean, Duron, Tarnovskaya, & Brem, 2003). In the U.S. population, half of the DM patients have diabetic peripheral neuropathy, and 11% of these suffer diabetic peripheral neuropathy pain (Low & Dotson 1998). However, the incidence of DFU pain while walking and at rest may rise up to 57% (Ribu et al., 2006). The DFU pain is associated not only with neuropathy but with peripheral vascular disease and infectious processes (Freedman et al., 2003; Sibbald, Armstrong, & Orsted, 2003). However, there is no definite evidence to clarify why some DFU patients suffer from pain but others do not.

Activation of mu-opioid receptor may alleviate acute or chronic pain via endogenous opioid peptides such as β-endorphin and endomorphins (Zadina, Hackler, Ge, & Kastin, 1997). Moreover, clinical observations reveal that interindividual variability in pain perception and varied sensitivity to opioid effects exist in human pain-relevant genes such as the opioid receptor mu 1 (*OPRM1*) gene. A number of single-nucleotide polymorphisms (SNPs) have been described for the opioid receptor (Bond et al., 1998; Grosch, Niederberger, Lotsch, Skarke, & Geisslinger, 2001). At nucleotide 118 of *OPRM1*, an adenine substitution by a guanine (A118G), which resulted in asparagine changing into aspartate at amino acid nucleotide 40, may play a major role in mediating the effects of opioids. In vitro, the A118G polymorphism seems to increase the binding affinity and potency of β-endorphin (Bond et al., 1998). Fillingim et al. (2005) demonstrated that patients with A118G polymorphism stand for more pressure pain and ischemic pain than the A118 common allele patients. This polymorphism may decrease response to specific nociceptive stimulation by modulation of nociceptive input (Lotsch, Stuck, & Hummel, 2006). The A118G polymorphism has not been studied with regard to its association with the painful DFU patients. Therefore, the purpose of this study was to determine the allelic frequency of the G118 variant in patients with painful DFU or with painless DFU by using the techniques of amplified DNA fragments of the involved gene, polymerase chain reaction–single-strand conformation polymorphism (PCR–SSCP), and sequenced DNA to clarify the role of A118G polymorphism of *OPRM1* in DFU pain.

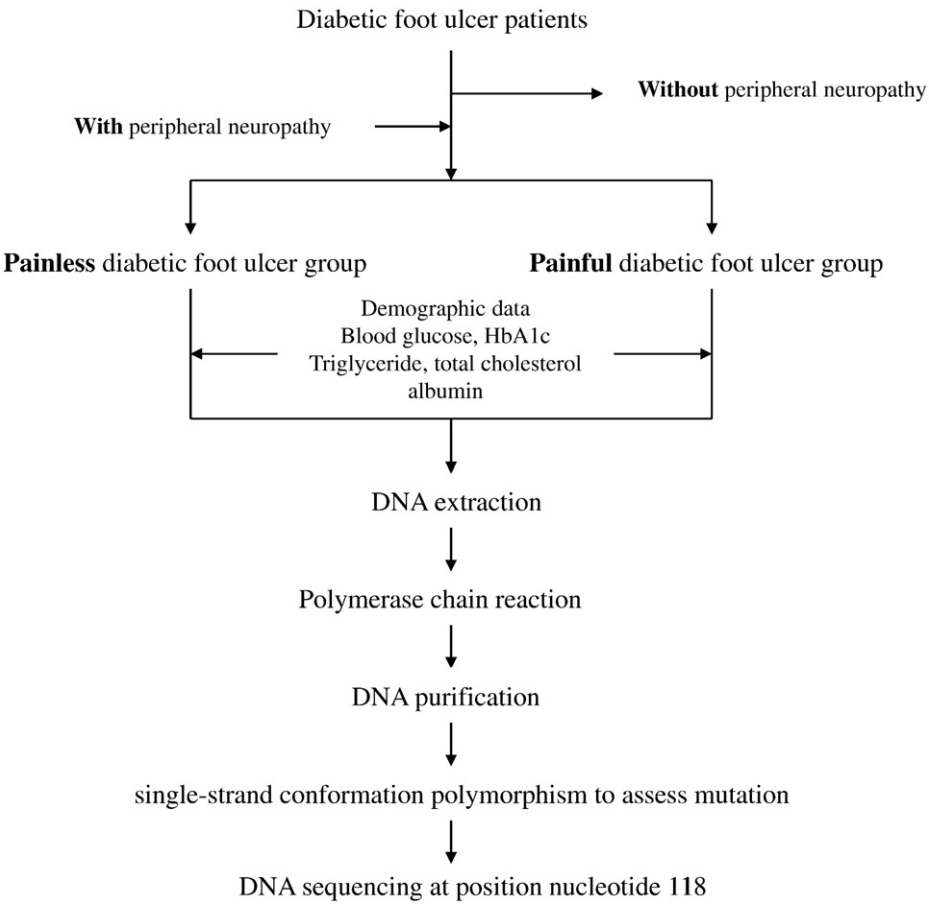

Fig. 1. Flowchart of this study.