Mephobarbital (MPB) is metabolized to 4'-hydroxy-MPB by cytochrome P450 2C19 (CYP2C19). We investigated the stereoselective pharmacokinetic disposition of MPB in the homozygous extensive metabolizers (homoEMs), heterozygous EMs (heteroEMs) and poor metabolizers (PMs) of CYP2C19 recruited from Japanese population. The mean area under the concentration-time curve (AUC) of R-MPB was greater in PMs than in EMs. The cumulative urinary excretion of 4'-hydroxy-MPB up to 24 hours postdose was 21-fold less in PMs than in EMs. However, one data point derived from a subject of heteroEM deviated from the other data of the heteroEMs for the plasma concentration of R-MPB and urinary excretion of 4'-hydroxy-MPB. The data suggest that this subject genotyped as 2C19*1/2C19*2, may carry a novel mutant allele of CYP2C19 in addition to CYP2C19*2. Therefore, we sequenced all nine exons and exon-intron junctions of CYP2C19 gene and found a novel single nucleotide polymorphism (SNP) in exon 9 (168946C>T), which induces an amino acid alteration (Arg442Cys). Since this SNP locates close to the heme-binding region of CYP2C19, it may result in the decrease in the catalytic properties of CYP2C19. This allele was newly designated as CYP2C19*16.