Supplementary Methods

Real time RT-PCR
Quantitative real-time PCR was performed using the Sybr green detection system with primer sets designed on Primer Express. Specific PCR product amplification was confirmed with gel electrophoresis. Transcript regulation was determined using the relative standard curve method per manufacture's instructions (Applied Biosystems). For each time point four samples of pooled tissue of two rats was analyzed.

In situ hybridization
Fresh frozen DRGs were cut at 14 µm, postfixed and acetylated. Riboprobes were obtained by in vitro transcription of cDNA and labeled with digoxigenin (Dig-labeling kit, Roche). Sections were hybridized with 200 ng/ml of sense or antisense probes in a prehybridization mix (5 x SSC, 50% formamide, 2 x Denhardt's, 500 µg/ml herring sperm DNA, 250 µg/ml yeast tRNA), hybridized with 200 ng/ml of sense or antisense probes in prehybridization mix, incubated with anti-Dig-alkaline phosphatase in 0.1 M maleic acid buffer, developed with NBT/BCIP/levamisole (Boehringer Mannheim), embedded in glycerol/gelatin or subjected to post in situ immunostaining. Primary antibodies: sheep Dig-AP 1:1000 (Roche), rabbit ATF-3 1:300 (SantaCruz). Blocking and antibody incubations in 1% blocking reagent (Roche) in maleic acid buffer.

Drug treatment
DAHP was dissolved in 1:1 polyethylene glycol (PEG400) and 1 x PBS, pH 7.4 (15 mg/ml) and administered i.p. or intrathecally (250 µg/kg/h, 5 µl/h). For all i.t. injections/infusions a spinal catheter (Recathco) was used. Infusions were done with an osmotic pump (Alzet). 6R-BH4 and neopterin in artificial cerebrospinal fluid were injected i.t. (10 µg, single 10 µl injection). N-acetyl-serotonin in 1 x PBS pH 7.4 containing 3% ethanol was delivered by i.t. infusion (100 µg/kg/h; 5 µl/h) or single i.p. injection (50 mg/kg). L-NAME (25 mg/kg, dissolved in 0.9% NaCl) was injected i.p. Control animals received the appropriate vehicle. All drugs were from Sigma-Aldrich.

Plasma and CSF concentrations of DAHP
Concentrations of DAHP were determined by LC/MS-MS on a tandem quadrupole mass spectrometer (PE Sciex API 3000; Applied Biosystems). Extraction by acetonitrile precipitation; chromatographic separation on a Nucleosil C18 Nautilus column (125 x 4 mm I.D., 5 µm particle size; 100 Å pore size). Mobile phase was acetonitrile:water (80:20%, v/v), and formic acid (0.1%, v/v). Flow rate 0.2 ml/min, injection volume 5 µl. DAHP eluted at 4.7 min. Mass spectrometer in positive ion mode, 5200 V, 400 m/z. The mass transition for the MRM was m/z 127→60. Quantification with Analyst software V1.1 (Applied Biosystems). The coefficient of variation over the calibration range of 10–4000 ng/ml was < 5%.

Chronic lumbar root pain study
Prior to the analyses, we specified a single primary endpoint, persistent leg pain over the first postoperative year after discectomy, as a reflection of ongoing neuropathic pain. Leg pain was assessed before surgery and at 3, 6 and 12 months after surgery by four items: Frequencies in the past week of "leg pain", and of "leg pain after walking", were rated as never (0 points), very rarely (1), a few times (2), about ½ the time (3), usually (4), almost always (5), and always (6). Improvements in "leg pain" or in "leg pain after walking" since surgery were rated as pain completely gone (0), much better (1), better (2), a little better (3), about the same (4), a little worse (5), and much worse (6). For each variable in each patient, we calculated an area-under-the-curve score for the first year and standardized the patients' AUC scores for each variable to z-scores which have a mean equal to 0 and standard deviation equal to 1. The primary pain outcome variable was the mean of these four z-scores. Genotype-phenotype associations for each SNP were sought by regression analysis using the equation: Individual pain score = (R1*number of uncommon alleles) + (R2*covariates) + error. (R1, R2 = regression coefficients). The covariates were a number of demographic, psychological and environmental factors, including sex, age, workman’s compensation status, delay in surgery after initial enrollment, and Short-Form 36 (SF-36) general health scale. Stepwise regression was applied to assess the association between pain scores and haplotypes by modeling pain scores as a function of all haplotypes generated by the 15 GCH1 SNPs and of relevant covariates. Only haplotypes with frequencies >1% were included in the model and were used as independent variables. If a haplotype was associated with a pain score that differed significantly from the average pain score (P < 0.05), phenotype-haplotype association analysis was performed by regression analysis using a similar model as described above for individual SNPs.
**Genotyping methods:**

*SNP markers:* The physical position and frequency of minor alleles (> 0.05) from a commercial database (Celera Discovery System, CDS, July, 2005) were used to select SNPs. 5’ nuclease assays (*vide infra*) was designed for fifteen *GCH1* SNPs. These panels of approximately equally-spaced markers covered the *GCH1* gene region plus 10 kb upstream and 6 kb downstream of the gene. Allele frequencies of all markers and their locations are shown in *Supplementary Table 2.*

*Genomic DNA:* Genomic DNA was extracted from lymphoblastoid cell lines and diluted to a concentration of 5 ng/µl. Two-µl aliquots were dried in 384-well plates.

*Polymerase Chain Reaction (PCR) amplification:* Genotyping was performed by the 5’ nuclease method using fluorogenic allele-specific probes. Oligonucleotide primer and probe sets were designed based on gene sequences from the CDS, July 2005. Primers and detection probes for each locus in each gene are listed in *Supplementary Table 3.*

Reactions were performed in a 5 µl volume containing 2.25 µl TE (Assays On Demand) or 2.375 µl TE (Assays By Design), 2.5 µl PCR Master Mix (ABI, Foster City, CA), 10 ng genomic DNA, 900 nM of each forward and reverse primer, and 100 nM of each reporter and quencher probe. DNA was incubated at 50 °C for 2 min and at 95 °C for 10 min, and amplified on an ABI 9700 device for 40 cycles at 92 °C (Assays on Demand) or 95 °C (Assays By Design) for 15 s and 60 °C for 1 min. Allele-specific signals were distinguished by measuring endpoint 6-FAM or VIC fluorescence intensities at 508 nm and 560 nm, respectively, and genotypes were generated using *Sequence Detection V.1.7* (ABI).

Genotyping error rate was directly determined by re-genotyping 25% of the samples, randomly chosen, for each locus. The overall error rate was < 0.005. Genotype completion rate was 0.99.

*Inference of haplotypes:* Haplotype phases – i.e. how the directly measured SNP alleles were distributed into two chromosomes in each patient – were inferred by the expectation-maximization (EM) algorithm (SAS/Genetics, Cary, North Carolina, USA). We had pre-specified that in the case where a gene had no common SNP proven to alter function, the primary analysis would compare the common haplotypes.