

Yan *et al.* - Supporting Online Material

Materials and Methods

Allele Expression Assay. Lymphoblastoid cell lines representing two genetically unrelated individuals from each of 48 CEPH reference families were obtained from the National Institute of General Medical Sciences repository maintained by the Coriell Institute for Medical Research. Cells were grown in RPMI with 10% FBS, and mRNA was isolated from 2×10^6 cells using the Amersham Pharmacia QuickPrep micro mRNA purification kit. Relative expression of each allele was determined as outlined in Fig. S1A. In brief, RT-PCR products from each allele of the gene of interest were distinguished using an ABI Prism SNaPshot Multiplex Kit and analyzed on a SpectruMedix SCE9610 Genetic Analysis system. Single base extension methods similar to those we used have been independently shown to yield highly quantitative results (*S1*). The data were converted to a relative allele expression using customized software. Sequences of the primers used for PCR amplification and SNP determination are available upon request.

Statistical Analysis and Validation. The fractional allelic experiment for each sample was determined through seven replicates. Prior to subsequent statistical analyses, obvious technical failures or statistical outliers were eliminated. In no case did this result in elimination of more than three replicates and on average resulted in elimination of one in every 25 data points. To validate our approach, we examined allelic expression of the *APC* tumor suppressor gene in CEPH individuals and in a familial adenomatous polyposis (FAP) patient previously shown to have decreased expression of one allele (*S2*). No significant variation in fractional allelic expression was observed in any of 17 heterozygous CEPH individuals tested when the data were analyzed using the MIXED procedure of the SAS system version 8.0 for repeated measurements. Specifically, this analysis revealed that none of the 17 individuals tested for expression of *APC* had a fractional allelic expression value that exceeded the 95% confidence interval for the mean. In contrast, the control FAP patient was well outside these limits (Fig. S1B).

Because no significant variation in expression of *APC* could be detected in these 17 individuals or in 24 individuals tested previously by a digital-PCR based approach (*S2*), we concluded that there was little genetic variation in *APC* expression and that *APC* could thereby be used to model analysis of other genes wherein the extent of variation was unknown. The analysis of the *APC* allelic expression ratios of the normal individuals described above yielded 95% confidence intervals ranging from 0.79 to 1.27. In the analysis of other genes, samples initially falling outside this 95% confidence interval (0.8 to 1.25) were selected for additional experiments. Any differences interpreted to represent variations in allelic expression was observed in multiple independent RNA samples and where possible, confirmed with an antisense primer. Using these criteria, we estimate that we were able to confidently identify variation when the differences between expression of the two alleles differed by more than 20% .

Haplotype and Pedigree Analyses. Microsatellite markers for haplotype analyses were obtained from Research Genetics and the amplified fragments were separated on a

SpectruMedix SCE9610 Genetic Analysis System. The distances from markers D4S1542, D4S2409, D4S2630 to *PKD2* are 1.4, 2.1 and 10.9 Mb, respectively; and distances from markers D2S345, D2S338, D2S336 to *Calpain-10* are 3.7, 4.3 and 5.7 Mb, respectively. Six of nine families proved uninformative after haplotype analyses. In five of these families, the spouse of the individual exhibiting an altered allelic expression ratio was homozygous for the SNP used to score expression. In one family showing variations in *FBN1* expression, altered allelic expression was detected in individuals from both the maternal and paternal sides of the pedigree, precluding unequivocal assignment of expression status in the offspring. The remaining three families showed patterns consistent with Mendelian inheritance of the altered expression.

Figure S1

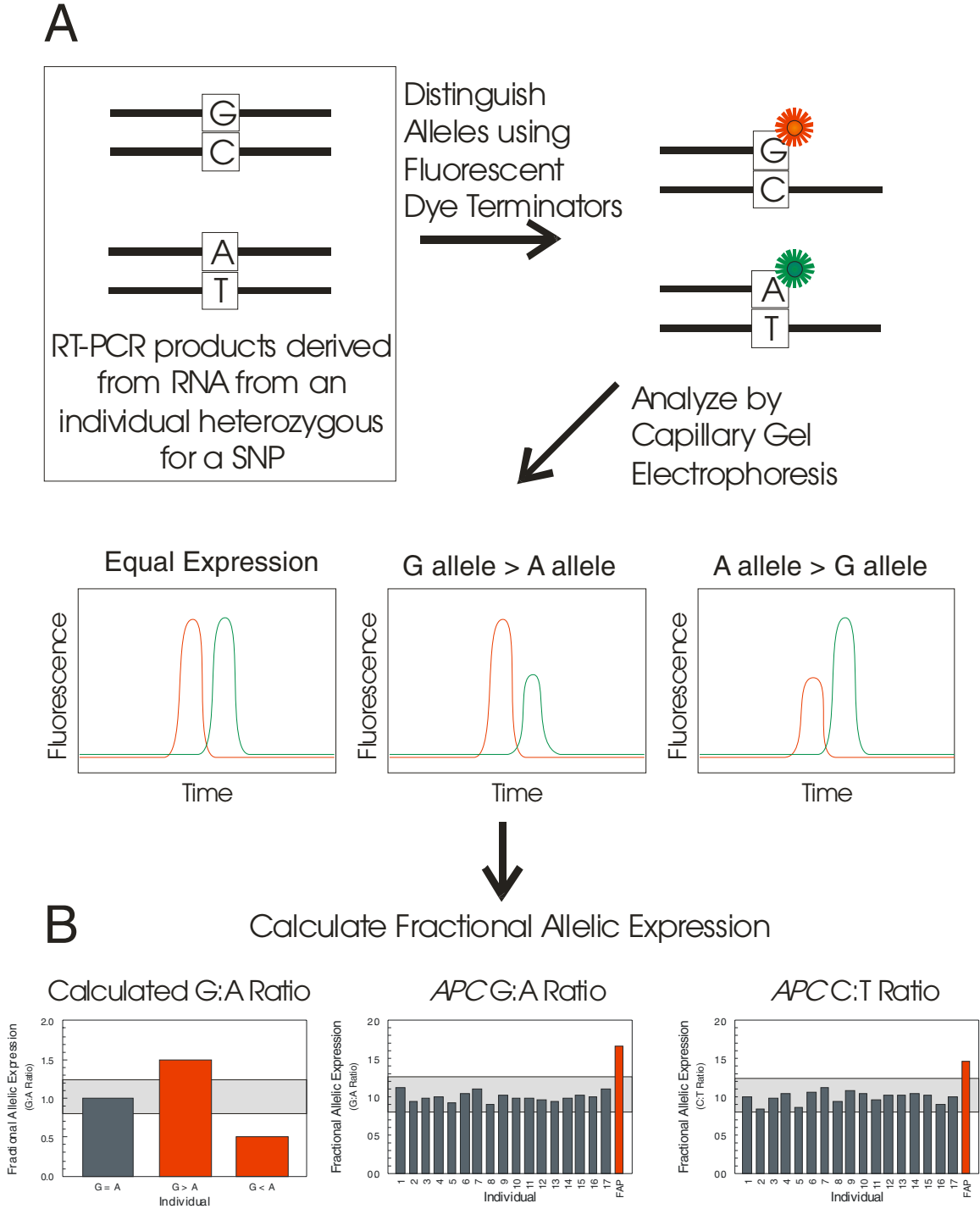


Fig. S1 Measuring allelic variation in gene expression. (A) Schematic of assay for fractional allelic expression showing key steps. See text for additional details. (B) Allelic expression analyses of *APC* in 17 CEPH individuals and an FAP Patient. The shaded box represents approximated 95% confidence interval and the red bars indicate individuals displaying significant variations.

References

S1. G. Matyas, C. Giunta, B. Steinmann, J.P. Hossle, and R. Hellwig, *Hum Mutat* **19**, 58-68 (2002).

S2. H. Yan *et al.*, *Nat Genet* **30**, 25-6 (2002).