Supplementary Table 1. Variants numbers in different QC stages. S1: SNP-set after genotyping rate, missing rate in samples and monopolymorphism removing. S2: remove non-loss-of-functional related SNPs from S1 dataset.

Supplementary Table 2. All nonsynomymous SNV in FGF6 exon regions and missense mutation prediction with different algorithms.

Supplementary Table 3. Real-time PCR Primers for FGF6 network validation

Supplementary Figure 1. Two-Site Power Calculations. Power calculations for a two-site disease model comparing the Armitage trend test of disease association at each site to a log-likelihood ratio test explicitly evaluating recessive diplotype effects. Baseline haplotype frequencies, case and control diploid sample sizes, and relative risk of disease-predisposing diplotypes parameters are shown. The initial haplotype frequencies (A1B1, A1B2, A2B1, A2B2) are presented. Different combinations of haplotypes are generated by generating recombination between the two sites and the results are presented in a collapsed manner through a single linkage disequilibrium metric. Hardy-Weinberg equilibrium of haplotypes/diplotypes in the general population is assumed. R is the relative risk of disease for recessive diplotypes compared to the remaining diplotypes. ncs and nct are the number of cases and controls, respectively. The type I error rate, adjusted for an exome-wide scan, was set to 2.5E-06 for all calculations.

Supplementary Figure 2. Minor Allele Frequency Distribution to PMRP dataset. Displayed is the histogram of the minor allele frequency (MAF) at each variant within the 10,000 PMRP subjects following removal of variants from the QC procedures.

Supplementary Figure 3. Quantile-Quantile Plot. Q-Q plot for the exome-wide, gene-based recessive diplotype scanning in hemochromatosis is shown. Numerous genes had no recessive diplotypes with putative functional alleles and therefore yielded P-values of 1. The two data points exceeding the confidence interval represented *HFE* and *FGF6*.

Supplementary Figure 4. Comparative genomic analysis and protein-protein interaction (PPI). The comparative genomic analyses revealed that *FGF6* evolved synchronously with other iron metabolism genes. **(A)** Main iron metabolism genes were collected and alignment was conducted to make the comparative genomic analysis together with *FGF6*. The earliest gene appearance over time was inferred by comparing species and corresponding evolution and appearance time was labelled. **(B)** Protein-protein interaction network was estimated by String (version 10.0)[SR1] using the highest confidence setting (confidence score>0.9).

Supplementary Figure 5. Total iron content in HFF-1 cells with increasing FGF-6 protein concentration. * $P<.05$; ** $P<0.01$. Results are the mean±SD of 3 observations in each experiment.

Supplementary Figure 6. *FGF6* mutation Plasmid Structures in the study. M1 (GAG->TAG) E172X, M2 (GAC->GTC) D174V and M3 (CGG -> CAG) R188Q

Supplementary Figure 7. Perls' stain reveals that *FGF6* loss-of-function nonsynonymous variants cause iron deposition. Perls' stain in HepG2 **(A)**, HCT-116 **(B)**, HCT-8 **(C)**, 786-O **(D)** and HFF-1 **(E)** in the presence of FAC differs among transfection by *FGF6* mRNA with wildtype and the identified variants R188Q, D174V and E172X.

Supplementary figure 8. *FGF6* loss-of-function nonsynonymous variants cause hepcidin downregulation and iron deposition in HFF-1. (A) Iron metabolism gene expression changes after the transfection by *FGF6* mRNA into HFF-1 with wildtype and the identified variants R188Q, D174V and E172X. **(B)** Total iron contents changes after the transfection by *FGF6* mRNA into HFF-1 with wildtype and the identified variants R188Q, D174V and E172X. **(C)** Ferritin protein level changes after the transfection by *FGF6* mRNA into HFF-1 with wildtype and the identified variants R188Q, D174V and E172X. **(D)** The densitometry data of Western blot for Ferritin protein were shown in the column chart. *$P<.05$; **$P<0.01$. Results are the mean±SD of three observations in one experiment.

Supplementary figure 9. Perls' stain in SSc and liver cancer. (A) Perls' stain was applied to evaluate the iron deposition in SSc skin tissues. Perls' stain was visualized by Nikon microscopy. The ratio of iron-positive stain areas to the total area was used to evaluate the iron deposition levels by Image J software. Arrows indicated positive stain area. **(B)** Perls' stain in liver cancer tissues. Perls' stain was visualized by Nikon microscopy. The ratio of iron-positive stain areas to the total area was used to evaluate the iron deposition levels by Image J software.

Supplementary figure 10. FGF-6 protein levels were different among normal, cancer and metastatic cells. (A) IHC of FGF-6 in normal hepatocytes and metastatic cells. The blue circle indicated normal liver tissue and the arrows indicated metastatic cells. (B) IHC of FGF-6 in non-metastatic liver cancer cells.

Supplementary figure 11. Relative transcription of iron metabolism genes with wildtype *FGF6* and three mutations. mRNA levels of *FGF6* and four iron metabolism genes are measured relative to *GAPDH* following transfection of vector, wildtype *FGF6* (WT), E172X *FGF6* (M1), D174V *FGF6* (M2), and R188Q *FGF6* (M3). Measurements were taken in three cell lines: 786-O, A498, and HCT-8.

Supplemental Reference

SR1. Szklarczyk, D. *et al.* STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res* **43**, D447-52 (2015).