## Figure legends

**Figure 1.** **Manhattan plot of the gene-based recessive diplotype association results.** The association *P*-value testing hemochromatosis association for each gene (y-axis) on different chromosomes is shown in alternating navy blue and green along the x-axis, with genes reaching experiment-wise significance for the gene-based analyses (experiment-wise  = 3.14 × 10−6) depicted in red.

**Figure 2.** Protein domain summarized by a previous FGF-6 functional study52. HBS: Heparin Binding Sites; FGFR-BR: FGFR-Binding Region.

**Figure 3.** **FGF-6 Active protein dosage effect on** intracellular **iron concentration.** Ferrozine assay was applied for the evaluation of total cell iron content in HepG2, 786-O, HCT-8, HCT -116 and HFF-1. 10μM FAC and 500μM ascorbate were added into the cell culture medians, respectively, with different concentration of FGF6 active protein. Control group was treated with ascorbate alone. After 48h incubation, cells were lysed and determined iron contents with ferrozine assay. **(A)** Total iron content in HepG2 cells. **(B)** Total iron content in 786-O cells. **(C)** Total iron content in HCT-8 cells. **(D)** Total iron content in HCT-116 cells. **(E)** Total iron content in HFF-1 cells. \* P＜.05; \*\* P＜0.01. Results are the mean±SD of 3 observations in 1 experiments.

**Figure 4.** ***FGF6* loss-of-function nonsynonymous variants cause hepcidin downregulation and iron deposition. (A)** Iron metabolism gene expression changes with FGF-6 active protein treatment in the cell culture media. **(B)** Iron metabolism gene expression changes with *FGF6* mRNA transfection in the cell culture media. **(C-E)** Iron metabolism gene expression changes after the transfection by *FGF6* mRNA into various cell types with wildtype and the identified variants R188Q, D174V and E172X. Cell lines: HepG2 are liver hepatocellular carcinoma cells, HCT-116 are ileocecal colorectal adenocarcinoma cells and HFF-1 are human normal skin fibroblasts. **(F-H)** Total iron contents changes after the transfection by *FGF6* mRNA into 3 cell types with wildtype and the identified variants R188Q, D174V and E172X. (I,G) Ferritin protein level changes after the transfection by *FGF6* mRNA into 3 cell types with wildtype and the identified variants R188Q, D174V and E172X. \* P＜.05; \*\* P＜0.01. Results are the mean±SD of 3 observations in 1 experiments.

**Figure 5. Perls’ stain and Ferritin expression. (A)** FGF-6 protein level was evaluated by immunohistochemistry assay (IHC) in skin tissues from SSc patients. **(B)** Perls’ stain was applied to evaluate the iron deposition in SSc skin tissues. **(C) I**mmunohistochemistry assay of Ferritin protein in SSc skin tissues. **(D)** Immunohistochemistry assay of FGF-6 protein in liver cancer tissues. (E) Perls’ stain was applied to evaluate the iron deposition in liver cancer tissues. (F) Immunohistochemistry assay of Ferritin protein in liver cancer tissues. Prussian blue staining was applied to evaluate the iron deposition in liver cancer tissues. FGF-6 expression level (Immunohistochemistry, IHC staining) in SSc tissues were counted ratio of positive stain cells per visual field, FGF-6 expression level in liver cancer tissues and ferritin expression levels were counted by average optical density (AOD) of positive signal in each sample. Iron staining 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.

**Figure 6. The proposed mechanism of FGF6 in the regulation of hepcidin expression and hepcidin-dependent iron.** Paracrine FGF6 interacts with FGFR with Heparin or heparan sulphate proteoglycan (HPSG) as the cofactor to initial FGF pathway (Nobuyuki itoh, JBC, 2011). Activated FGFRs have the ability to phosphorylate specific tyrosine residues and activate STAT3 pathway (Turner, Nature Review Cancer 2010). Iron overload, and inflammation could positively regulate hepcidin by BMP/Smad pathway (Ivana, JCI, 2007) and inflammatory IL-6/STAT3 pathways (Thomas, JCI, 2010 and Diedra, blood, 2006). However, loss-of-function mutation of FGF6 will silence FGF6-FGFR pathway and cause low expression of hapcidin which will decrease the inhibition of irons transfer from intestinal cell to blood. In the SSc patients, IL-6 was increased so that hapcidin will be positively regulated which the iron releasing will be suppressed caused higher iron levels in skin cells.

## Supplementary Figure legends

**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 explicity evaluating recessive diplotype effects. Baseline haplotype frequencies, case and control diploid sample sizes, and relative risk of disease-predisposing diplotypes parameters are shown. The of 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 1 error rate, adjusted for an exome-wide scan, was set to 2.5E-06 for all calculations.

**Supplementary Figure 2.** **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 3. **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)51 using the highest confidence setting (confidence score>0.9).

**Supplementary figure 3. Perls’ stain reveals that *FGF6* loss-of-function nonsynonymous variants cause iron deposition.** Perls’ stain of various cell types (HepG2, HCT-116, HCT-8, 786-O and HFF-1) in the presence of FAC differs among transfection by FGF6 mRNA with wildtype and the identified variants R188Q, D174V and E172X.

**Supplementary figure 2. Gray-scale value of ferritin protein after FGF6 mRNA transfection.** Western blot was performed to detect the protein level of ferritin after transfection by FGF6 mRNA with wildtype and the identified variants R188Q, D174V and E172X. Gray-scale value were measured by Image J software.

**Supplementary figure 3. Normal body iron metabolism (A) and iron metabolism when FGF6 mutated in various tissues. (A)** FGF6 can promote the expression of hepcidin which is a critical regulator of iron metabolism. High hepcidin concentration can reduces iron absorption and impairs iron release from stores. and impaires and dcrease the level of ferritin, while low hepcidin concentration can increase iron absorption and iron delivery from storage sites. **(B)** Nonsynonymous mutation of FGF6 can lead body more sensitive to inflammation and cancer. Functional deficiency of FGF6 impaired increase of hepcidin and decrease of ferritin and finally lead to iron overload.