## Figure legends

**Figure 1.**

**Figure 2.**

**Figure 3.** **FGF-6 Active protein regulates hepcidin-dependent iron uptake.** 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 added into the cell culture medians, respectively, with different concentration of FGF6 active protein. Control group 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, the protein concentration was 250ng/ml, the incubation time was 24h. **(B)** Iron metabolism gene expression changes with *FGF6* mRNA transfection in the cell culture media after 24h. **(C-D)** Iron metabolism gene expression changes after the transfection by *FGF6* mRNA into cells 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. **(E-F)** Total iron contents changes after the transfection by *FGF6* mRNA into 2 cell types with wildtype and the identified variants R188Q, D174V and E172X in the presence of FAC for 48h. **(G-H)** Ferritin protein level changes after the transfection by *FGF6* mRNA into 2 cell types with wildtype and the identified variants R188Q, D174V and E172X in the presence of FAC for 48h. The gray-scale value of ferritin peotein level were shown in column chart. \* 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 and the average optical density (AOD) were quantified by Image J software. **(B)** IHC of Ferritin protein in SSc skin tissues and the AOD were quantified by Image J software. **(C)** IHC of FGF-6 protein in liver cancer tissues and the AOD were quantified by Image J software. **(D)** IHC of Ferritin protein in liver cancer tissues and the AOD were quantified 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. Minor Allele Frequency Distribution to PMRP dataset.** Distribution of minor allele frequency (MAF) to PMRP dataset, which was applied for gene-based recessive diplotype scanning in hemochromatosis analysis.

**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)51 using the highest confidence setting (confidence score>0.9).

**Supplementary figure 5. 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 6. 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 3 observations in 1 experiments.

**Supplementary figure 7. 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 8. FGF6 protein levels were different among normal, cancer and metastatic cells. (A)** IHC of FGF6 in normal hepatocytes and metastatic cells. The blue circle indicated normal liver tissue and the arrows indicated metastatic cells. (B) IHC of FGF6 in non-metastatic liver cancer cells.