Effect of Fluorosis on liver cells of VC deficient and wild type mice

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Running title: Effect of Fluorosis on cells from VC deficient and wild type mice

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ABSTRACT

For decades, mouse and other rodents have been used for study of oxidative or related studies such as the effect of fluoride. It is known that rodents normally synthesize their own vitamin C (VC) due to the presence of a key enzyme in ascorbic acid synthesis, l-gulono-lactone-γ-oxidase (Gulo), while humans do not have the capacity of VC synthesis due to the deletion of most part of the GULO gene. The spontaneous fracture (*sfx)* mouse recently emerged as a model for study of VC deficiency. We investigated the effect of fluoride on liver cells from wild type Balb/c and *sfx* mice. We found that reduction of SOD, GPx and CAT activities were reduced in both wild type and *sfx* mice; however, the amount of reduction in the *sfx* cells is more than that in Balb/c cells. In addition, while both cells increased MDA, the increase in the *sfx* cells is greater than that in Balb/c cells. Gene networks of *Sod*, *Gpx* and *Cat* in the liver of humans and mice are also different. Our study suggests that reaction to fluoride in Vitamin C deficient mice might be different from that of wild type mice.

Key Words: Fluorosis, Mouse, Oxidative, Vitamin C, Liver cell

1. Introduction

Human and mouse genes are 99% the same; however, a few important differences, including the Vitamin synthesis, should have been paid much attention, particularly for the biomedical research. Our study intends to explore the answer whether the wild type mouse model is the right choice for the study of fluoride and, to certain extend, the oxidative stress. To date, many evidence strongly indicates that fluorosis is closely related to oxidative stress [1-5]. Furthermore, study of oxidative stress using mouse model has been widely accepted. An important issue related to mouse model is that vitamin C (VC) was suggested to reduce oxidative stress as one of antioxidants [4, 6-7]. It is known that mice normally synthesize their own vitamin C due to the presence of a key enzyme in ascorbic acid synthesis, l-gulono-lactone-γ-oxidase (*Gulo*), which is present in the liver. However, this enzyme is deficient in humans. Because mice can synthesize VC, it is not considered an essential dietary component for this species, and therefore VC is not usually added to mouse chow. In contrast, humans depend entirely on dietary supplementation for their VC.

For decades, the utilization of mouse or other rodents for study of oxidative or related studies such as fluoride as the animal model has never been challenged. As of August 5th, 2013, using the two key words “fluoride” and “mouse”, we found 1674 publication from PubMed. When we restricted the search for the year of 2013 only, we found 46 publications. Using key words ‘oxidative mouse”, we found 26606 publications from PubMed. Among these publications 2008 are from 2013, but none of them apparently are using the VC deficiency mice. Our recently study indicates there are different gene expression levels between wild type mice and VC deficient *sfx/sfx* mice, which were supplied with sufficient quantities vitamin C [8-9]. One key question is whether the differential expressions between wild type and VC deficient mice lead them to react differently to environmental stimulations such as by oxidative toxic components.

To explore the difference in vitro between cells from *sfx* and wild type mice, we investigated the effect of fluoride on liver cells from wild type and *sfx* mice. We assume that using liver cells from *sfx/sfx* mouse of fluorosis may allow us to have a better understanding of the mechanism of fluorosis without the disturbance of vitamin C. Thus, we intended to provide the direct evidence that wild type mice are not a good model to study human oxidative pathways. Particularly, we examined the differences in oxidative stress indexes between cells from wild type Babl/c and *sfx/sfx* mice.

2. Materials and methods

2.1. Cells culture

Hepatocytes were isolated from Balb/c and *sfx/sfx* mice with a body weight range of 20–35 g using the two-step hepatic portal vein perfusion technique [10]. Hepatocytes were cultured on rat tail collagen type I at a density of 5×10^5 cells/ml. Primary hepatocytes were maintained in a hepatoZYME-SFM Medium (Invitrogen, USA), supplemented with 200 U/mL penicillin, 100 μg/mL streptomycin (Sigma-Aldrich, USA), and 10% fetal bovine serum (Mediatech Inc., VA, USA) at 37˚C in a humidified atmosphere of 95% air/5% CO_2. The medium was changed after optimal attachment. Medium contains RPMI-1640 Medium (Hyclone, Utah,USA), supplemented with 200 U/mL penicillin, 100 μg/mL streptomycin, and 2% fetal bovine serum. In addition, the medium was introduced 12.5uM of Vitamin C and contained NaF at final concentrations of 0, 0.25, 0.5, 1, 2, 4, 8 mM, respectively. The cells were incubated for 1, 2, 4, 6, 12, 24, 48 and 72 h. All experiments were performed in triplicate.

2,2. Cell Growth and Viability assay

Hepatocytes were cultured in 96-well plates at a density of 2×10^4 cells/well. After allowing the cells to adhere, they were treated for 1, 2, 4, 6, 12, 24, 48 and 72 h with the indicated concentrations of Vitamin C and NaF. WST-8 (Cell Counting Kit-8/CCK-8, Sigma-Aldrich, MO, USA) assays were performed to quantify LS8 cell viability after exposure to the different concentrations of Vitamin C and NaF, respectively. The absorbance was measured at 450 nm using an Absorbance Microplate Reader (SpectraMax 340PC384,US). Five samples were analyzed and the mean value was calculated for each NaF concentration and time point.

2.3. Protein preparation and determination

Hepatocytes were cultured in 25 cm^2 flasks at a density of 2×10^6 cells/flask. After NaF treatment, the hepatocytes were harvest by the rubber policeman, then resuspended in 50 mM Tris buffer containing 0.5% Triton X-100, pH 8.0, and lysed by freeze thawing. Lysates were centrifuged at 6,000 × g for 15 min at 4°C. The supernatant obtained was

used for the SOD, CAT and GPx activities measurements. The supernatant was then removed and stored on ice. The protein content of the supernatant was determined using Thermo Scientific NanoDrop 2000 spectrophotometers (Thermo Fisher Scientific-NanoDrop products, Wilmington, Delaware USA).

2.4. SOD assay

Superoxide dismutase (SOD) activity was measured using the SOD determination kit – WST (Sigma-Aldrich, Buchs, Switzerland). As an inhibition activity, the SOD activity was quantified by measuring the developed color at 450 nm using the Absorbance Microplate Reader(SpectraMax 340PC384,US). All operations are following the manufacturer's instructions. The SOD activity was calculated as an inhibition rate percentage and expressed as units (SOD activity (units)=SOD activity(inhibition rate)/(1-SOD activity(inhibition rate.

2.5. CAT assay

Hepatocytes catalase (CAT) activity was determined using the OxiSelect™ Catalase Activity Assay Kit (Colorimetric method) (Cell Biolabs, San Diego, CA, USA). It records the decomposition of H_2O_2 by decreasing the absorbance at 520 nm. One unit of CAT activity is equal to the amount of enzyme(s) that decomposed 1.0 µmole of H_2O_2 per minute at 25°C. The CAT activity was calculated and expressed as U per mg protein.

2.6. GPx assay

Glutathione Peroxidase (GPx) activity was measured with the Glutathione Peroxidase assay kit (Cayman Chemical, Ann Arbor, MI, USA) in accordance with the manufacturer's instructions. Measurement of GPx activity was performed according to the Colorimetric method that is based on the monitoring of the oxidation of NADPH per minute at 25°C. The reaction rate can be determined using the NADPH extinction coefficient of 0.00373 μM^{-1} after adjusting for the path length of the solution in the well (0.6 cm). The absorbance was measured at 340 nm using the Absorbance Microplate Reader(SpectraMax 340PC384,US) and the concentration was expressed in nmoles GPx /min/mg protein.

2.7. Measurement of MDA

Hepatocytes were suspended at 1 x 10^7 cells/mL in PBS containing butylated hydroxytoluene for malondialdehyde (MDA). The hepatocytes were sonicated in phosphate-buffered saline containing butylated hydroxytoluene for MDA. The MDA in the whole homogenate were measured using the Oxiselect TBARS assay kit containing thiobarbituric acid-reactive substances (Cell Biolabs, San Diego, CA, USA). All standards and samples were assayed in duplicate in accordance with the manufacturer's instructions. The developed color was read at 532 nm using the Absorbance Microplate Reader (SpectraMax 340PC384, US). TBARS formation was expressed as μM TBARS 10^6 cells $^{-1}$.

2.8. Association of Gulo with oxidative genes

In order to understand the relationship between *Gulo* and genes of Glutathione Peroxidase, Superoxide dismutase and Hepatocytes catalase in liver, we examined the gene network among *Gulo* and three oxidative genes. Because gene network is built upon the expression of genes in multiple strains, we were not able to use the *sfx* mice (a single strain) with that of Balb/c for it. Therefore, we took the advantage of available gene expression data of multiple mouse strains and a human population in GeneNetwork (http://www.genenetwork.org/webqtl/main.py#). We examined the association of gene expression between *Gulo* and three oxidative stress genes in this study using gene expression profiles of mouse recombinant inbred strains generated using Affymetirx Array M430A by investigator at UCLA (Bennett et al., 2010] and of the Human Liver Cohort [11-12] which includes 427 human liver samples on a comprehensive gene expression microarray. The data has deposited at GeneNetwork.

2.9. Statistical Analysis

All results were expressed as mean ± SEM. The data were analyzed for statistical significance using the Student's t test. P-values less than 0.05 were considered statistically significant. All results were presented with histograms in Microsoft Excel.

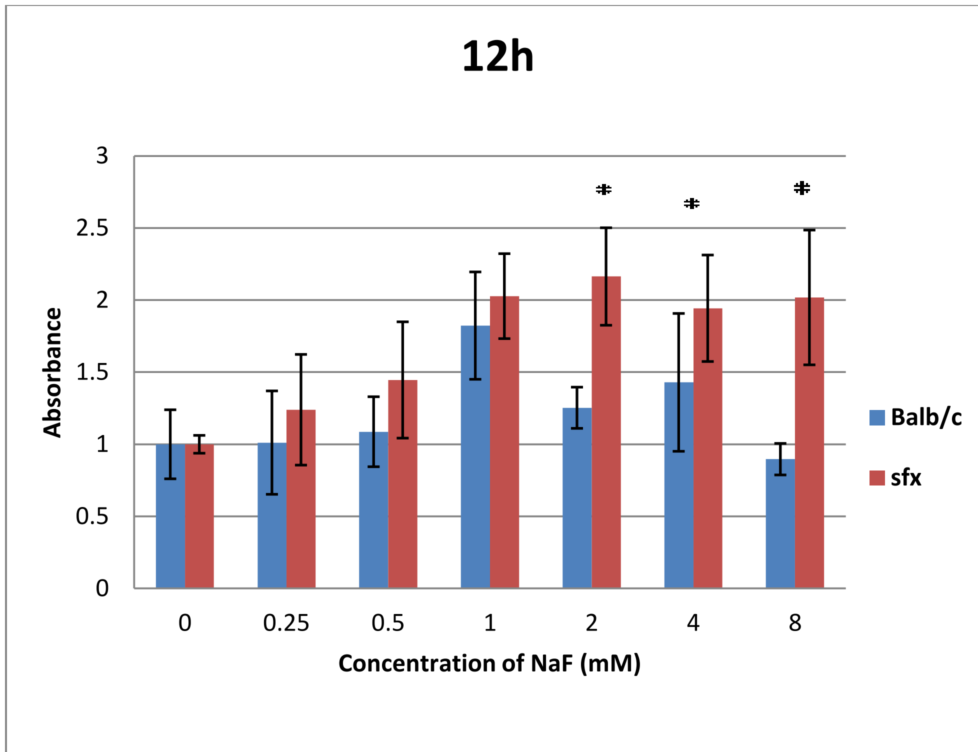
3. Results and discussion

3.1. The viabilities of hepatocytes from Balb/c and sfx/sfx mouse with Vitamin C

Cell viability was evaluated by the WST-8 assay. We first examined the viabilities of hepatocytes after treatment with Vitamin C. We intended to identify the concentrations of Vitamin C, which led to the same cell viability of hepatocytes from *sfx/sfx* and Balb/c mice. The results suggest that the cell viability of hepatocytes in the two types of mice is consistent at 12.5µm vitamin C from 1 h-72 h, at each time point (data not shown). Accordingly, we used 12.5um vitamin C in the late experiments.

3.2. The viabilities of hepatocytes from Balb/c and sfx/sfx mouse after treatment with NaF

Cell viability test results of the hepatocytes from Balb/c and *sfx* mice for NaF concentrations after 12, 24, 48 hrs were shown in histogram in Figure 1. In this experiment, the control groups of two types are calculated respectively as reference in the cell viabilities of all experimental groups. In 48 hours, because the viabilities of the control group of two types decreased with different degrees compared to 24 hours, the percentage of cell viabilities increased in some experimental groups. In this case, cell viability data showed improvement in 48 hours.


12h
3
2.5
2
1.5
1
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Absorbance
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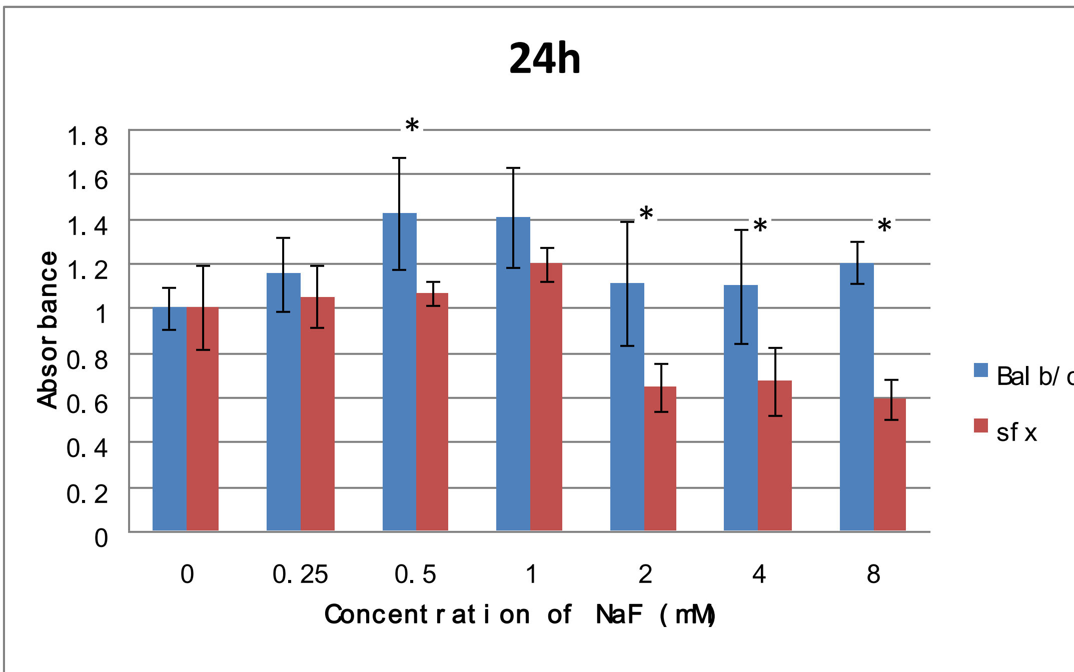
2
4
8
Concentration of NaF (mM)
Balb/c
sfx

*


24h
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
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Absorbance
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Concentration of NaF (mM)
Balb/c
sfx

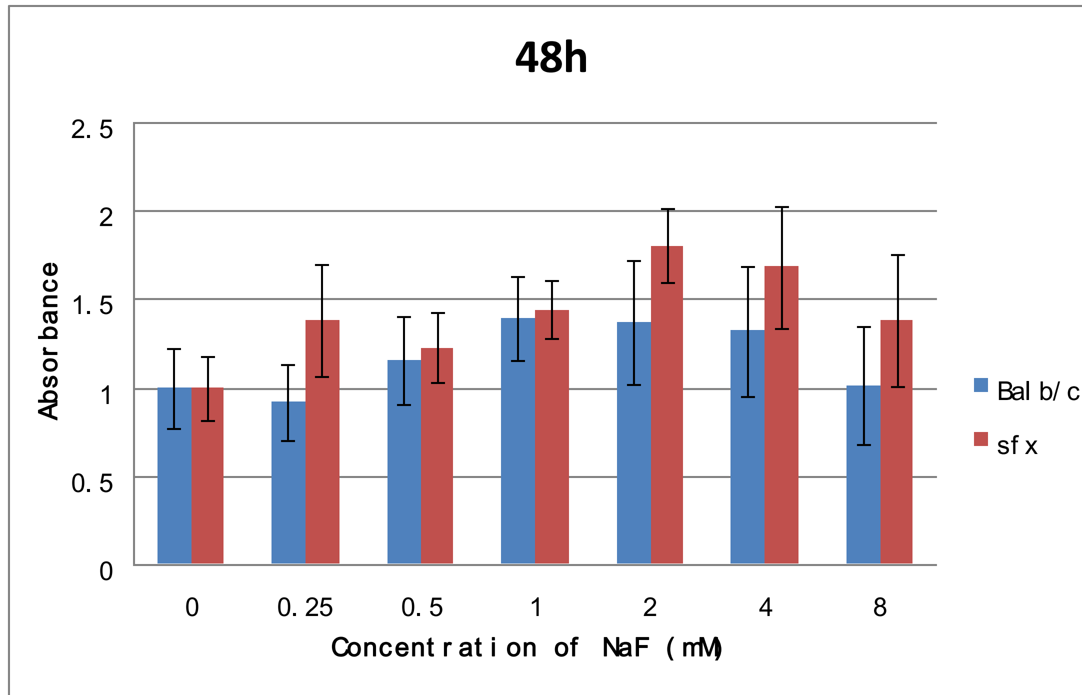

Figure 1. The viabilities of hepatocytes at three time points from Balb/c and *sfx/sfx* mouse after treatment with NaF. X axis: Concentration of NaF (mM); 3' UTR. Y axis: Absorbance.

The results show that the cell viability in two types of cells had a significant difference with higher concentration at 12 and 24 hrs. However, the cell viability between *sfx* and Babl/c between these two time points showed opposite results. At 12 hrs, the cell viability of 2, 4, and 8 mM of *sfx* mice is much better than that of Balb/c, with P values of 0.0005, 0.09, and 0.0008, respectively. In contract, at 24 hrs, the cell viability of 2, 4, and 8 mM of *sfx* mice is much less than that of Balb/c, with P values of 0.007, 0.01, and 5.77846E-06, respectively. This data seemingly suggest that there is a difference in the time-sequential of the response to Naf between cells from *sfx* and wild type.

In addition, at 48 h, the cell viability did not show a significant difference between those two types of cells, with P values of 0.04, 0.15, and 0.13 at concentration of 2, 4, and 8 mM, respectively. Again this may be the net result of opposite reactions at 12 and 24 hrs between cells from *sfx* and wild types.

3.3. Effects of NaF on Antioxidant Status

We measured the activities of Superoxide dismutase (SOD), Glutathione peroxidase (GPx) and Catalase (CAT) of hepatocytes from cells of Balb/c and *sfx* mice treated with 0.5 mM NaF for 48 hours. The results show that the SOD, GPx, and CAT activity are different between the control group and the NaF group in each of the two types of hepatocyte. Moreover, the differences between NaF treated and the control in *sfx* and wild type cells are also different, respectively (Fig. 2). The main biological roles of SOD, GPX and CAT are to protect the organism from oxidative damage. SOD is an enzyme that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. Thus, they are an important antioxidant defense in nearly all cells exposed to oxygen. The biochemical function of GPx is to reduce lipid hydroperoxides and to reduce free hydrogen peroxide in water. CAT catalyzes the decomposition of hydrogen peroxide in water and oxygen.

Figure 2A

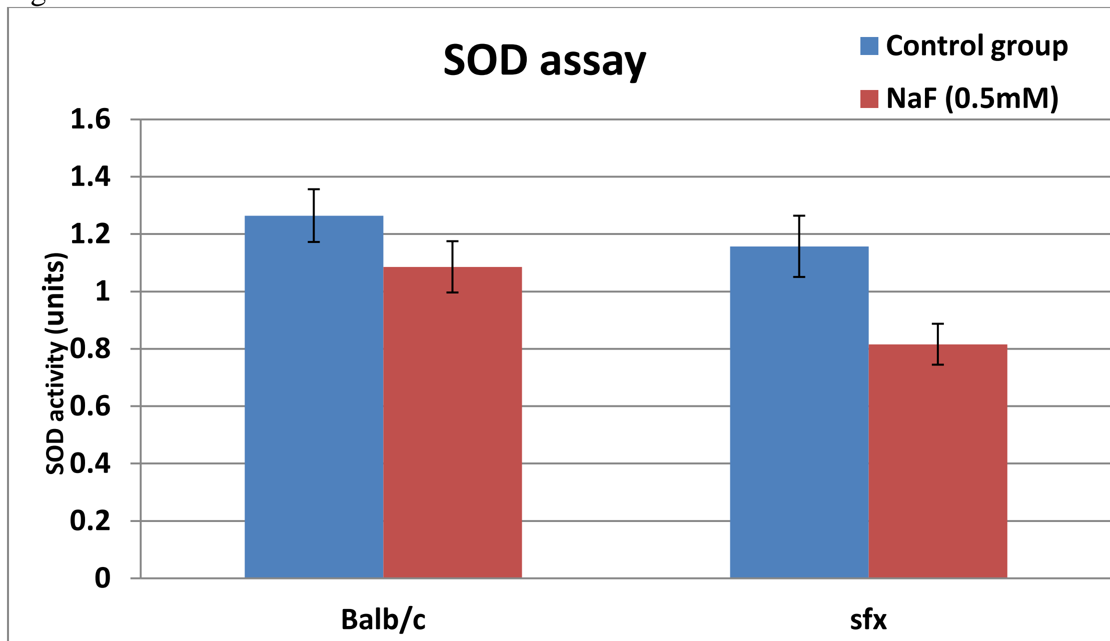

Figure 2B

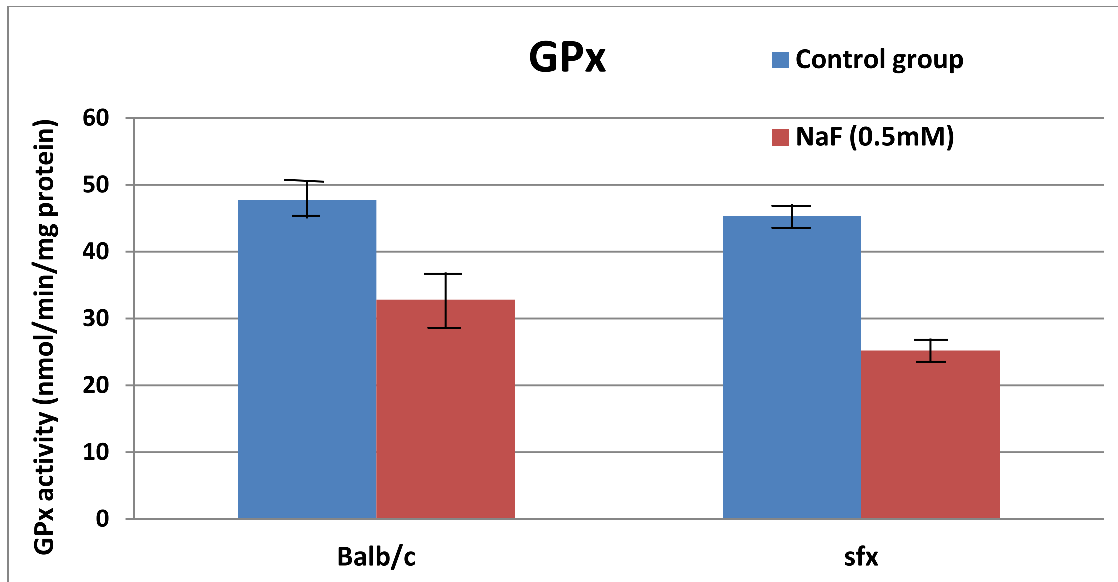

Figure 2C

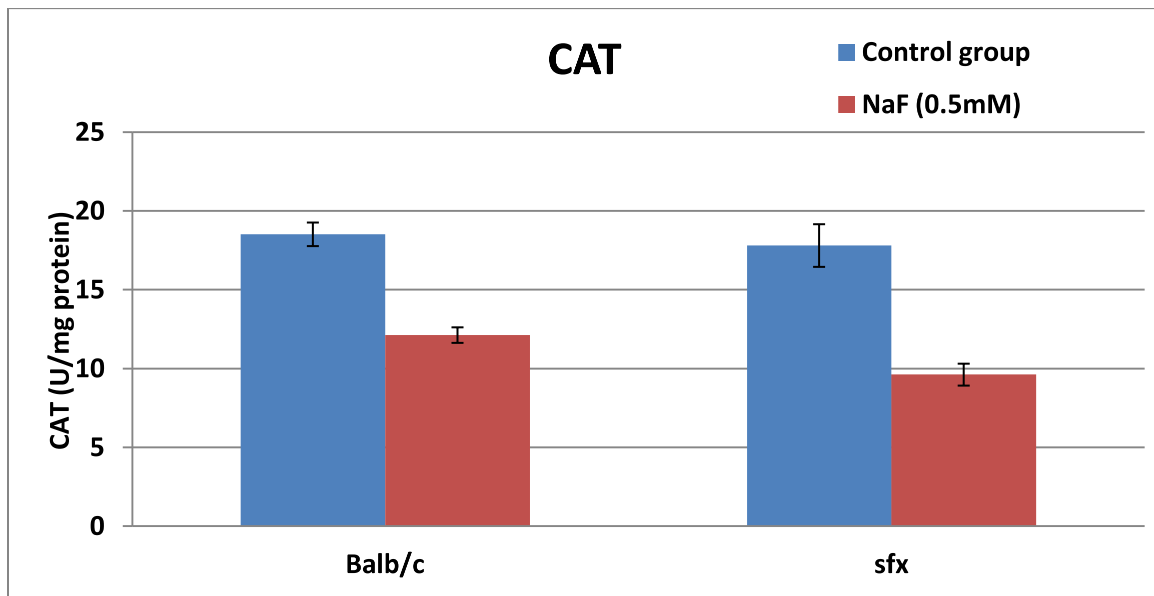

Figure 2D Effects of NaF on Lipid Peroxidation

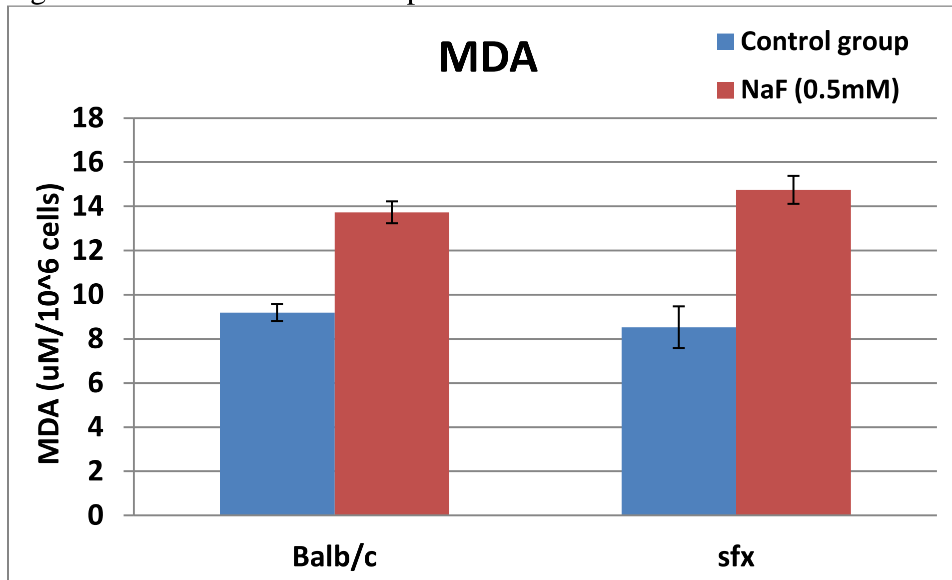

Figure 2. Effects of NaF on Antioxidant Status. X axis: cells from different mice in treatment. Y axis: units of enzyme activity. **2A**. SOD activity was measured using the SOD determination kit. The SOD activity was calculated as an inhibition rate percentage and expressed as units (SOD activity (units)=SOD activity(inhibition rate)/(1-SOD activity(inhibition rate). **2B**. CAT activity was determined using the OxiSelect™ Catalase Activity Assay Kit One unit of CAT activity is equal to the amount of enzyme(s) that decomposed 1.0 μmole of H_2O_2 per minute at 25°C. The CAT activity was calculated and expressed as U per mg protein. **2C**. GPx activity was measured with the Glutathione Peroxidase assay kit. The absorbance was measured at 340 nm using the Absorbance Microplate Reader(SpectraMax 340PC384,US) and the concentration was expressed in nmoles GPx /min/mg protein. **2D**. Effects of NaF on Lipid Peroxidation. The MDA in the whole homogenate were measured using the Oxiselect TBARS assay kit. The developed color was read at 532 nm using the Absorbance Microplate Reader.TBARS formation was expressed as μM TBARS 10^6 cells $^{-1}$.

In Balb/c cells, the activity of SOD in NaF (0.5mM) treated hepatocytes is lower than that in control cells (Fig. 2A). The P value is 0.022. In *sfx* cells, the activity of SOD in NaF (0.5mM) treated cells is significantly lower than that in control cells, with P value of 0.0002. The difference in the net reduction of SOD activity between cells from Balb/c

and *sfx* is 0.022. Thus, reduction of SOD activity in the *sfx* cells is much greater than that in Balb/c cells.

NaF (0.5mM) treatment showed the greatest effect on the Gpx activity in hepatocytes (Fig. 2B). In Balb/c cells, the activity of GPx in NaF (0.5mM) treated cells is much lower than that in control cells. The P value is 8.10652E-06. In *sfx* cells, the activity GPx in NaF (0.5mM) treated cells is also significantly lower than that in control cells, with P value of 7.66305E-06. The difference in GPx activity between hepatocytes from Balb/c and *sfx* reached to a significant level, with P value of 0.0016. Thus, amount of reduction of GPx activity in the *sfx* cells is much greater than that in Balb/c cells.

Changes of CAT activity are similar to that of SOD (Fig. 2C). In Balb/c hepatocytes, the activity of CAT in NaF (0.5mM) treated cells is much lower than that in control cells. The P value is 1.51688E-05. In *sfx* cells, the activity CAT in NaF (0.5mM) treated cells is also significantly lower than that in control cells, with P value of 2.31606E-08. The difference in CAT activity between cells from Balb/c and *sfx* also shows a significant level, with P value of 0.0297.

These data raises the question whether the study of the fluoride and oxidative stress or Vitamin C relevant genes should use the *Gulo* deficient mouse model. Since the wild type mouse has the endogenous Vitamin C synthesize gene, which humans have lost during evolution. The result from wild type mice may not be as applicable to humans as that from *sfx* mice.

3.4. Effects of NaF on Lipid Peroxidation

Lipid peroxidation product MDA was measured in the NaF treated and wild type Balb/c hepatocytes. As shown in Fig. 2D, MDA content was significantly higher in NaF treated hepatocytes compared to control groups from both Balb/c and wild type controls, with P values of 8.56273E-06 and 1.25082E-07, respectively. Moreover, the amount of increase of MDA in NaF treated *sfx* hepatocytes is greater than that in balb/c cells, with P value of 0.0095. Thus, the amount of net changes of MAD in hepatocytes shows opposite result in comparing with that of SOD, GPx and CAT.

MDA is the end product of lipid peroxidation considered as a sensitive index to assess lipid peroxidation. The increased MDA and decreased SOD, GPx and CAT in *sfx*

mice agree each other. The hepatocytes MDA levels of NaF groups were significantly increased, indicating the presence of enhanced lipid peroxidation due to NaF injury. Moreover, there are significant differences between cells from Balb/c and *sfx* mouse in MDA quantity changes (in NaF group), indicating that there are differences between the two models in reactions to fluorosis.

3.5. Association of Gulo with GPx Cat, and Sod genes in wild type mice in human population

From GeneNetwork, we identified 18 probes for mouse genes, *Gpx1, Gpx3, Gpx4, Gpx5, Gpx1, Gpx7, Gpx8, Gulo, Cat, Sod1, Sod2,* and *Sod3*. From data of human liver cohort, we identified 15 probes for genes GPX1, GPX3, GPX4, GPX5, GPX1, GPX7, GPX8, CAT, SOD1, SOD2, and SOD3. Because humans do not have *Gulo* gene, we first compared Spearmen Rank Correlation with without *Gulo* in mice and humans separately. A third analysis was done with mouse genes including *Gulo*.

The correlation of expression levels among major oxidative genes are opposite between mice and humans (Supplementary Table s1 and s2). For example, 1) in mice, the expression level of Catalase is positively correlated to the expression level of *Sod2*, with R value of 0.678. In humans, the expression of Catalase is positively correlated to that of GPX4 and SOD1, with R values of 0.5 and 0.8, respectively. Furthermore, Catalase is strongly negatively correlated to SOD2, with R value of -0.7. 2). In mice, *Sod1* did not show strong correlation to other genes, while *Sod2* showed positive correlation with *Gpx1* and *Gpx4* with R values of 0.5 for both. In humans, SOD1 is negatively correlated to GPX1, GPX3 and SOD2, with R values of -0.51, -0.51, and -0.57.

We next examined whether expression of *Gulo* is correlated to the expression of any of those genes in mice. We found that the expression level of *Gulo* is negatively correlated to one gene, the Gpx3, with R value of -0. 558 (Fig. 3). Furthermore, the expression of *Gulo* has a weak positive correlation with that of catalase, *Sod1* and *Sod2* (Fig. 3).

Figure 3A: *Gulo* and Gpx3

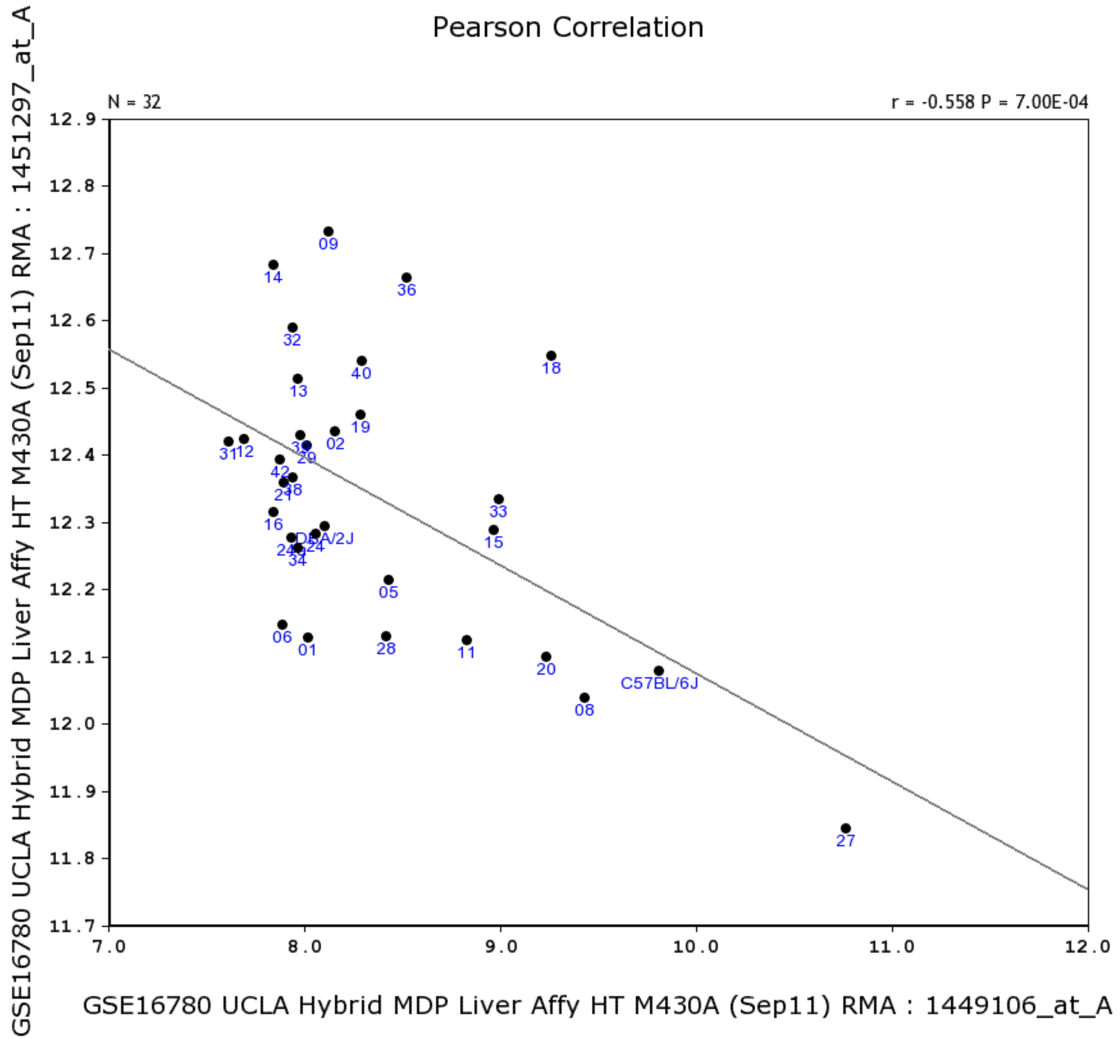

Figure 3B. *Gulo* Vs Cat

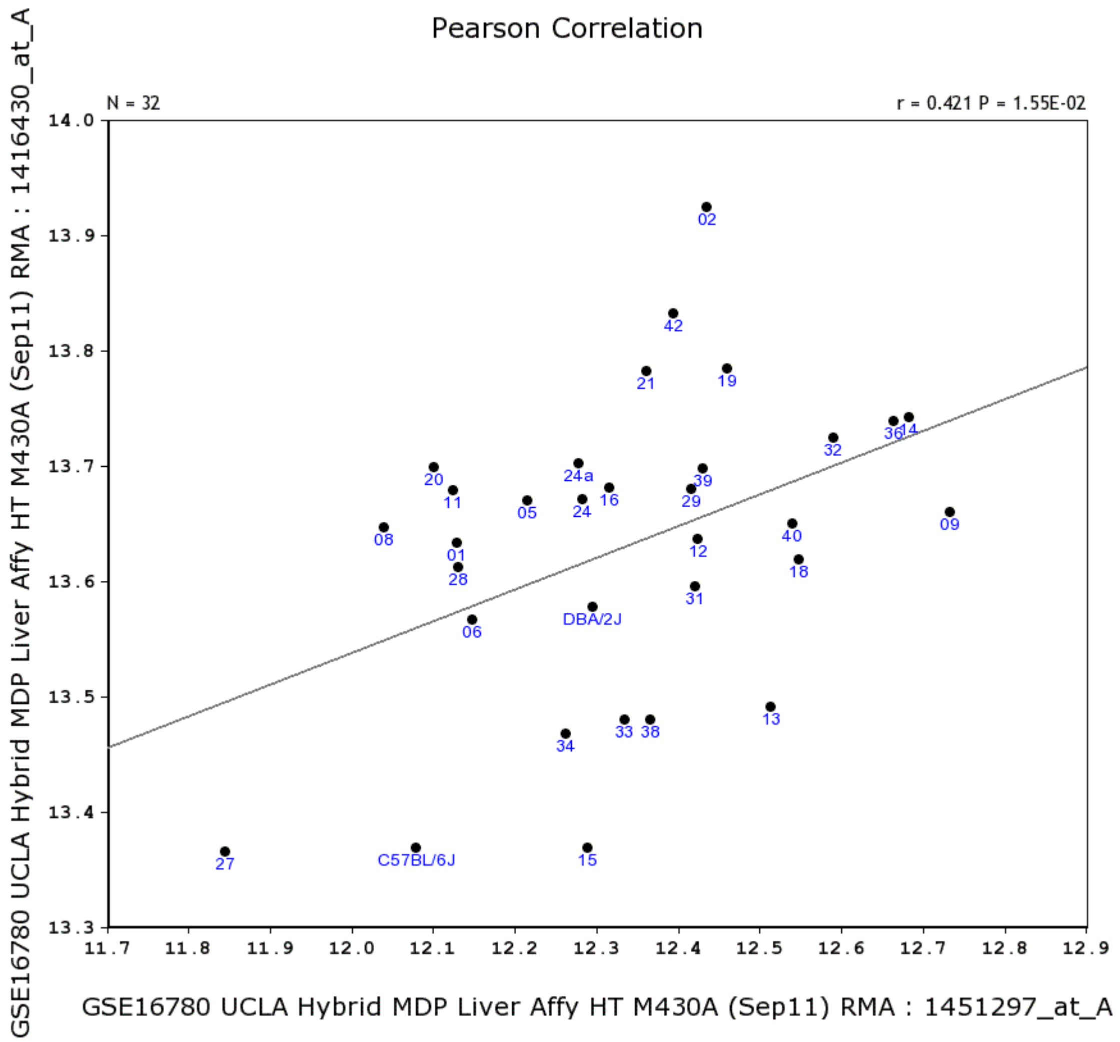

Figure 3C. *Gulo* vs Sod1

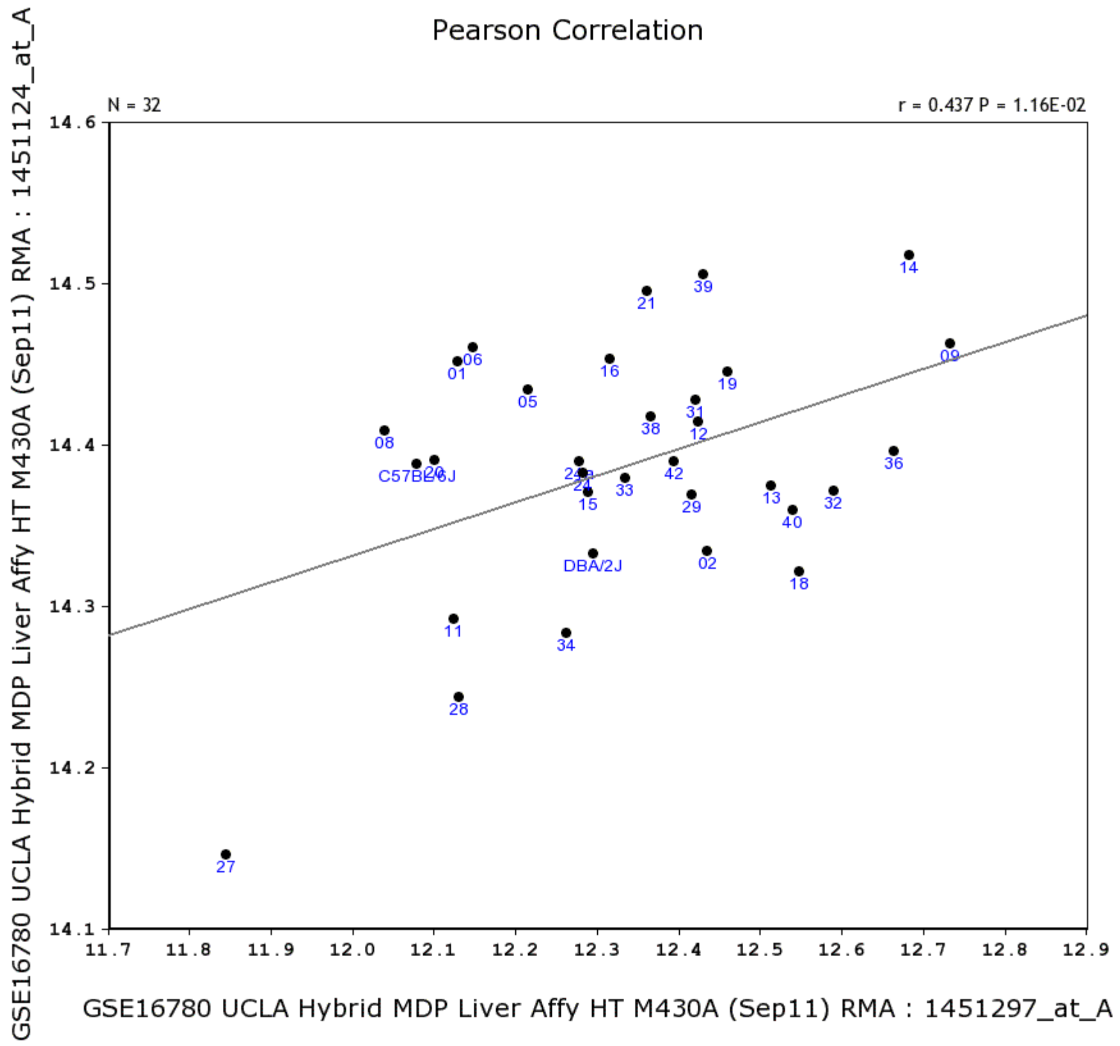
Pearson Correlation
N = 32
r = 0.437 P = 1.16E-02
GSE16780 UCLA Hybrid MDP Liver Affy HT M430A (Sep11) RMA : 1451124_at_A
GSE16780 UCLA Hybrid MDP Liver Affy HT M430A (Sep11) RMA : 1451297_at_A

Figure 3D. *Gulo* vs Sod2

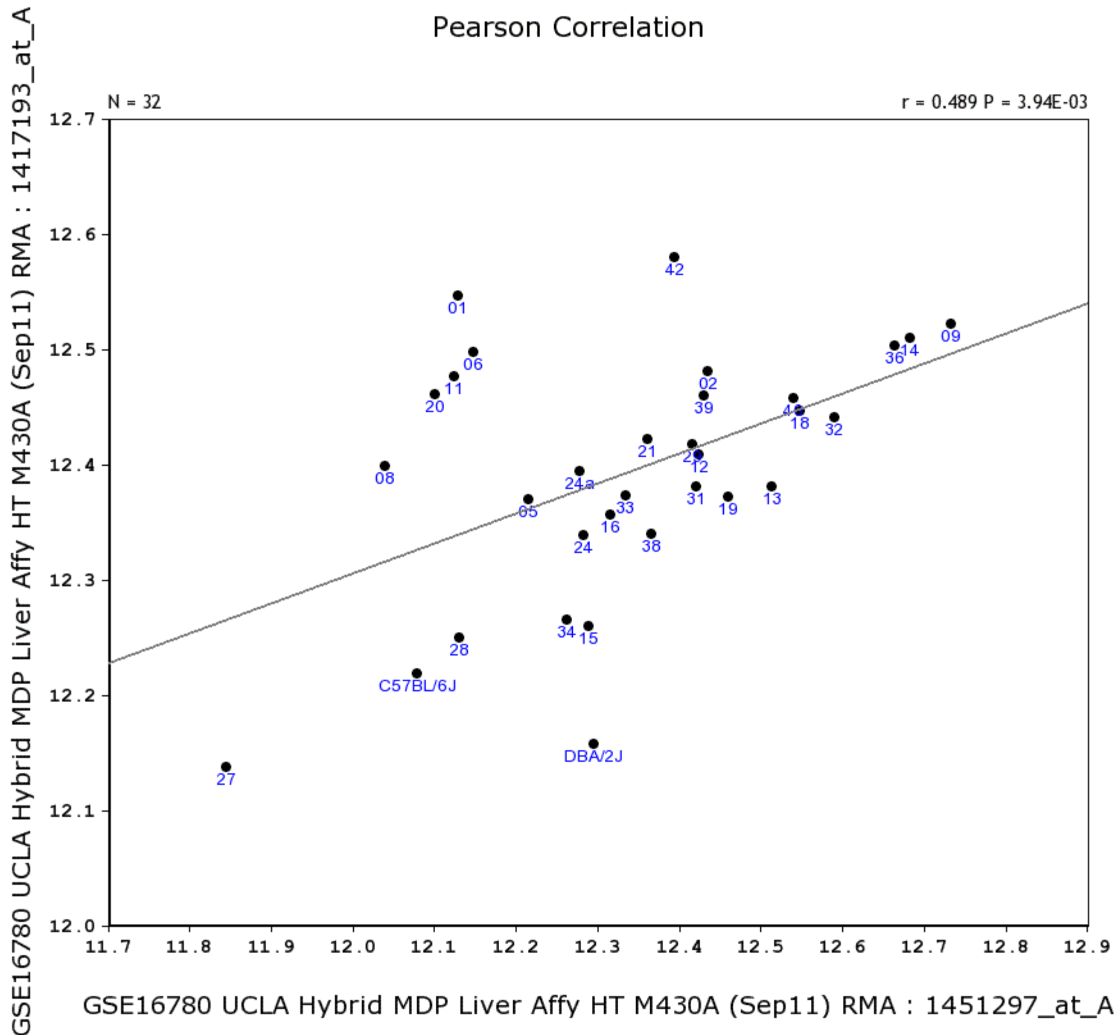

Figure 3. Correlation between expression level of *Gulo* and Gpx3, Cat, Sod1, and Sod2 in mouse liver. Gene expression data are from GSE16780 UCLA Hybrid MDP Liver Affy HT M430A (Sep11) RMA Database. **3A**. X axis: Gpx3: glutathione peroxidase 3; 3' UTR. Y axis: *Gulo*: gulonolactone (L-) oxidase (ascorbic acid biosynthesis; mid 3' UTR . **3B.** X axis: *Gulo* mid 3' UTR. Y axis: Cat mid 3' UTR **3C.** X axis: *Gulo* mid 3' UTR; **Y** axis: *Sod1* first four exons and 3' UTR **3D.** X axis: *Gulo* mid 3' UTR; **Y** axis: *Sod2* last three exons and proximal 3' UTR

We finally constructed the gene network for both mice and humans. As shown in Figure 4, the gene networks between mice and humans are significantly different. In mice, four pairs of positive and negative associated genes forms the core of the network. They are *Gulo* and *Gpx3, Sod3* and *Sod2, Gpx4* and *Gpx1*, and *Cat* and *Sod2*. In humans, the network core is composed with CAT and SOD1 with several GPX genes. Thus, the expression of *Gulo* at least partially influences the expression of those oxidative genes in mice which potentially lead to the difference in the pathways between none-*Gulo* humans and mice with *Gulo* gene.

Figure 4A. mouse gene network

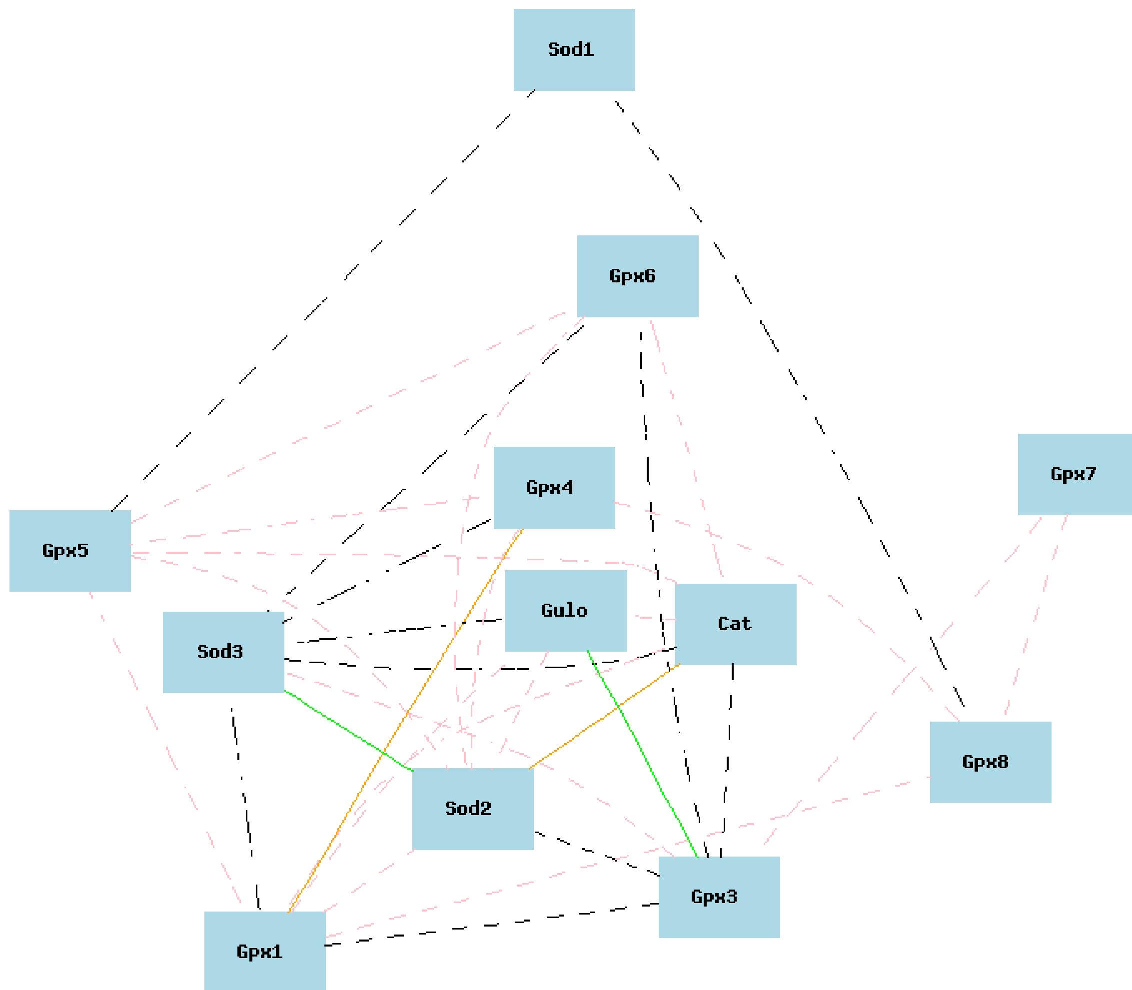

Figure 4B. Human gene network

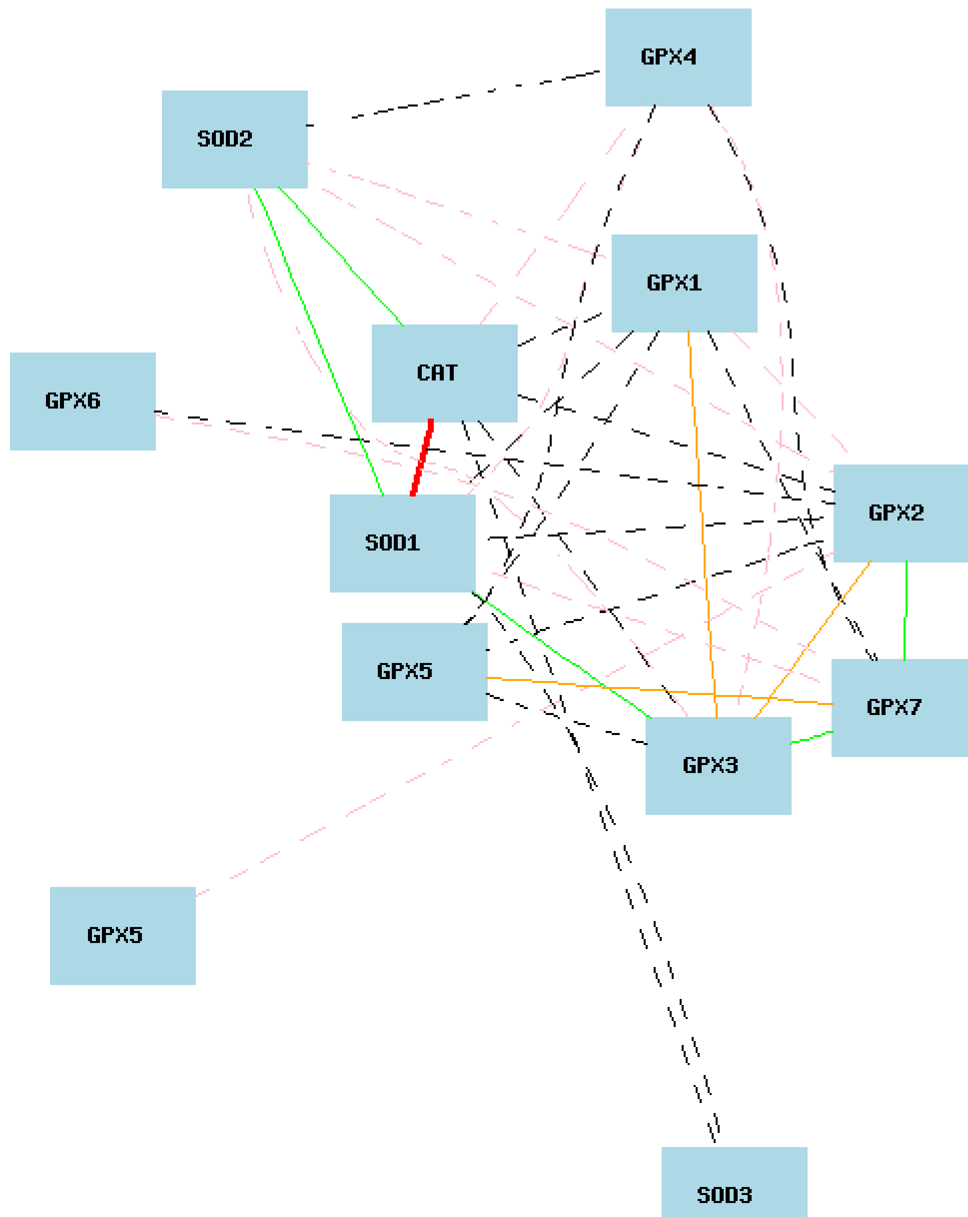

Figure 4. Gene networks of mice and humans. The 13 nodes in the graph show the selected traits. All nodes are displayed. The 47 edges between the nodes, filtered from the 78 total edges and drawn as curves, show Pearson correlation coefficients greater than 0.2 or less than -0.2. The graph's canvas is 40.0 by 40.0 cm, and the node labels are drawn

with a 10.0 point font, and the edge labels are drawn with a 10.0 point font. Figure 4A gene network of *Gulo* and oxidative genes in mice. Figure 4B gene network of oxidative genes in human liver.

The gene networks in this study are composed by using mouse RI strains and human population. Because the *Gulo* deficient mouse is a single strain, the gene network in mouse strains of lack of *Gulo* has not been constructed. Future study on the impact of *Gulo* deficient on the oxidative network in vivo will enhance our understanding of detailed molecular mechanism of *Gulo* deficient oxidative pathway in mice.

4. Conclusions

Our data show that there are significant differences in SOD, GPx and CAT activity and MDA production when treated with fluoride between liver cells of Balb/c and the *Gulo* deficient *sfx* mice, indicating that there are differences between wild type and Vitamin C deficient mice for the study of fluorosis. Comparison of gene co-expressions of these oxidative genes between mice and humans suggest that their molecular pathways are different. Taken together, we hypothesized that the wild type mice have inevitable shortcomings as a model to study human oxidative relevant disorders, as it has ignored the role of vitamin C in the oxidative pathway.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Author contributions: W.G., W.W. and Y.J. designed experiments and analyzed data. W.W. and X. L. performed the cell experimental work. Y. J., Y. M. and F. Z. collected the animal tissues. L. W. and W. G. analyzed the gene network. Y. J. W. G. and D. S. conceived and managed the project. W. W., Y. J., W. G. and D. S. contributed to manuscript preparation. All authors contribute to manuscript finalization.

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Supplementary Table s1. Spearman rank correlation among mouse three oxidative genes (Mouse without Gulo)

Spearman Rank Correlation (rho)

Pearson r

	Trait1	Trait2	Trait3	Trait4	Trait5	Trait6	Trait7	Trait8	Trait9	Trait10	Trait11	Trait12	Trait13	Trait14	Trait15	Trait16	Trait17
Trait 1: GSE16780_UCLA_ML0911::1416429_a_at_A Cat on Chr 2 @ 103.294184 Mb catalase distal 3' UTR	***n*** 32	0.752 32	0.141 32	-0.324 32	0.093 32	0.209 32	0.189 32	0.404 32	-0.044 32	-0.072 32	0.451 32	-0.144 32	0.514 32	0.366 32	0.178 32	-0.318 32	-0.367 32
Trait 2: GSE16780_UCLA_ML0911::1416430_at_A Cat on Chr 2 @ 103.29421 Mb catalase mid 3' UTR	0.754 32	***n*** 32	0.237 32	-0.289 32	-0.040 32	0.035 32	0.231 32	0.180 32	0.107 32	-0.010 32	0.349 32	-0.044 32	0.589 32	0.464 32	0.237 32	-0.023 32	-0.172 32
Trait 3: GSE16780_UCLA_ML0911::1460671_at_A Gpx1 on Chr 9 @ 108.241871 Mb	0.152 32	0.247 32	***n*** 32	-0.144 32	0.475 32	0.537 32	0.253 32	-0.120 32	0.137 32	0.179 32	0.366 32	-0.135 32	0.410 32	0.416 32	-0.162 32	0.043 32	-0.359 32

glutathione peroxidase 1 exons 1 and 2 and proximal 3' UTR																	
Trait 4: GSE16780_UCLA_ML0911::1449106_at_A Gpx3 on Chr 11 @ 54.723291 Mb glutathione peroxidase 3 3' UTR	-0.445 32	-0.478 32	-0.335 32	***n*** 32	-0.073 32	-0.096 32	-0.085 32	-0.268 32	0.254 32	-0.006 32	-0.471 32	0.177 32	-0.238 32	-0.173 32	-0.343 32	0.079 32	0.238 32
Trait 5: GSE16780_UCLA_ML0911::1451695_a_at_A Gpx4 on Chr 10 @ 45.595286 Mb glutathione peroxidase 4 last five exons	0.016 32	0.004 32	0.654 32	-0.125 32	***n*** 32	0.666 32	0.220 32	0.248 32	-0.102 32	0.512 32	0.387 32	-0.289 32	0.194 32	0.258 32	0.076 32	0.084 32	-0.403 32
Trait 6: GSE16780_UCLA_ML0911::1456193_x_at_A Gpx4 on Chr 10 @ 45.595578 Mb glutathione peroxidase 4 last exon and 3' UTR	0.170 32	0.122 32	0.704 32	-0.195 32	0.804 32	***n*** 32	0.429 32	0.115 32	-0.018 32	0.376 32	0.441 32	-0.262 32	0.328 32	0.458 32	-0.242 32	-0.125 32	-0.151 32
Trait 7: GSE16780_UCLA_ML0911::1420698_at_A Gpx5 on Chr 13 @ 21.378334 Mb glutathione peroxidase 5 distal 3' UTR	0.137 32	0.248 32	0.326 32	-0.017 32	0.301 32	0.423 32	***n*** 32	0.089 32	-0.061 32	0.009 32	0.113 32	-0.219 32	0.261 32	0.398 32	-0.361 32	0.173 32	0.212 32

Trait 8: GSE16780_UCLA_ML0911::1452135_at_A Gpx6 on Chr 13 @ 21.410881 Mb glutathione peroxidase 6	0.417 32	0.329 32	-0.017 32	-0.255 32	0.148 32	0.043 32	0.245 32	***n*** 32	-0.264 32	0.188 32	0.366 32	-0.182 32	0.153 32	0.049 32	0.139 32	-0.387 32	-0.283 32
Trait 9: GSE16780_UCLA_ML0911::1417836_at_A Gpx7 on Chr 4 @ 108.073058 Mb glutathione peroxidase 7	-0.223 32	-0.037 32	0.046 32	0.270 32	-0.099 32	-0.058 32	0.021 32	-0.076 32	***n*** 32	0.349 32	-0.422 32	-0.023 32	0.199 32	0.161 32	0.185 32	0.238 32	0.280 32
Trait 10: GSE16780_UCLA_ML0911::1424099_at_A Gpx8 on Chr 13 @ 113.833243 Mb glutathione peroxidase 8 last 2 exons and proximal 3' UTR	-0.296 32	-0.186 32	0.239 32	0.014 32	0.472 32	0.429 32	-0.078 32	-0.012 32	0.462 32	***n*** 32	0.125 32	-0.235 32	0.124 32	0.220 32	0.226 32	0.241 32	0.007 32
Trait 11: GSE16780_UCLA_ML0911::1451124_at_A Sod1 on Chr 16 @ 90.223027 Mb superoxide dismutase 1, soluble first four exons and 3' UTR	0.433 32	0.376 32	0.503 32	-0.555 32	0.428 32	0.490 32	0.008 32	0.269 32	-0.362 32	0.107 32	***n*** 32	-0.363 32	0.397 32	0.401 32	0.124 32	-0.261 32	-0.500 32
Trait 12: GSE16780_UCLA_ML0911::1435304_at_A Sod1 on Chr 16 @ 90.22488 Mb superoxide dismutase 1, soluble antisense in intron 3 and exon 4	-0.028 32	-0.052 32	-0.087 32	0.092 32	-0.187 32	-0.151 32	-0.213 32	-0.116 32	-0.097 32	-0.224 32	-0.212 32	***n*** 32	-0.113 32	-0.203 32	-0.053 32	-0.034 32	0.103 32

Trait 13: GSE16780_UCLA_ML0911::1417193_at_A Sod2 on Chr 17 @ 13.206311 Mb superoxide dismutase 2, mitochondrial last three exons and proximal 3' UTR	0.500 32	0.678 32	0.467 32	-0.426 32	0.328 32	0.449 32	0.307 32	0.325 32	0.024 32	0.076 32	0.549 32	-0.124 32	***n*** 32	0.906 32	0.114 32	-0.134 32	-0.355 32
Trait 14: GSE16780_UCLA_ML0911::1448610_a_at_A Sod2 on Chr 17 @ 13.208027 Mb superoxide dismutase 2, mitochondrial last exon and proximal 3' UTR	0.356 32	0.522 32	0.525 32	-0.310 32	0.422 32	0.586 32	0.433 32	0.214 32	0.039 32	0.205 32	0.514 32	-0.206 32	0.909 32	***n*** 32	0.025 32	-0.020 32	-0.249 32
Trait 15: GSE16780_UCLA_ML0911::1417194_at_A Sod2 on Chr 17 @ 13.20865 Mb superoxide dismutase 2, mitochondrial	0.219 32	0.200 32	-0.094 32	-0.295 32	0.049 32	-0.153 32	-0.237 32	0.314 32	0.204 32	0.168 32	0.246 32	-0.066 32	0.244 32	0.123 32	***n*** 32	0.140 32	-0.216 32
Trait 16: GSE16780_UCLA_ML0911::1417634_at_A Sod3 on Chr 5 @ 52.759404 Mb superoxide dismutase 3, extracellular	-0.401 32	-0.011 32	0.022 32	-0.081 32	0.082 32	-0.105 32	0.193 32	-0.281 32	0.141 32	0.207 32	-0.225 32	-0.244 32	-0.115 32	-0.045 32	-0.057 32	***n*** 32	0.346 32
Trait 17: GSE16780_UCLA_ML0911::1417633_at_A Sod3 on Chr 5 @ 52.760191 Mb superoxide dismutase 3, extracellular	-0.362 32	-0.326 32	-0.400 32	0.295 32	-0.407 32	-0.276 32	0.163 32	-0.260 32	0.082 32	-0.055 32	-0.465 32	0.084 32	-0.542 32	-0.409 32	-0.402 32	0.400 32	***n*** 32

Supplementary Table s2. Spearman rank correlation of three oxidative genes among humans (Human liver)

Spearman Rank Correlation (rho)

Pearson r

	Trait1	Trait2	Trait3	Trait4	Trait5	Trait6	Trait7	Trait8	Trait9	Trait10	Trait11	Trait12	Trait13	Trait14	Trait15
Trait 1: HLC_0311::10023826334 CAT on Chr 11 @ 34.493607 Mb catalase	***n*** 427	-0.488 427	-0.473 427	-0.458 427	0.516 427	0.077 427	-0.149 426	0.152 426	0.008 427	-0.336 427	-0.415 427	0.800 426	0.849 427	-0.712 427	-0.237 427
Trait 2: HLC_0311::10023814607 GPX1 on Chr 3 @ 49.394608 Mb glutathione peroxidase 1	-0.438 427	***n*** 427	0.467 427	0.495 427	-0.093 427	-0.361 427	0.195 426	-0.063 426	-0.146 427	0.302 427	0.340 427	-0.535 426	-0.519 427	0.391 427	-0.058 427
Trait 3: HLC_0311::10023809881 GPX2 on Chr 14 @ 65.405871 Mb glutathione peroxidase 2 (gastrointestinal)	-0.359 427	0.463 427	***n*** 427	0.332 427	-0.073 427	-0.306 427	0.207 426	-0.174 426	-0.134 427	0.263 427	0.417 427	-0.330 426	-0.366 427	0.337 427	0.032 427
Trait 4: HLC_0311::10023805643 GPX3 on Chr 5 @ 150.408554 Mb	-0.388 427	0.500 427	0.616 427	***n*** 427	-0.019 427	-0.233 427	0.285 426	-0.037 426	-0.181 427	0.127 427	0.050 427	-0.430 426	-0.443 427	0.440 427	0.180 427

glutathione peroxidase 3 (plasma)															
Trait 5: HLC_0311::10023815372 GPX4 on Chr 19 @ 1.106787 Mb glutathione peroxidase 4 (phospholipid hydroperoxidase)	0.459 427	-0.022 427	0.139 427	0.215 427	***n*** 427	-0.273 427	-0.157 426	-0.091 426	-0.376 427	-0.303 427	-0.379 427	0.385 426	0.437 427	-0.451 427	-0.200 427
Trait 6: HLC_0311::10023813546 GPX5 on Chr 6 @ 28.502728 Mb glutathione peroxidase 5 (epididymal androgen-related protein)	0.066 427	-0.398 427	-0.401 427	-0.366 427	-0.264 427	***n*** 427	-0.076 426	0.149 426	0.612 427	0.098 427	0.129 427	0.168 426	0.091 427	-0.096 427	0.129 427
Trait 7: HLC_0311::10023819620 GPX5 on Chr 6 @ 28.502728 Mb glutathione peroxidase 5 (epididymal androgen-related protein)	-0.088 426	0.097 426	0.207 426	0.179 426	-0.017 426	-0.004 426	***n*** 426	0.131 425	0.022 426	0.102 426	0.059 426	-0.030 425	-0.061 426	0.230 426	-0.162 426
Trait 8: HLC_0311::10025911877 GPX6 on Chr 6 @ 28.471072 Mb glutathione peroxidase 6 (olfactory)	0.139 426	-0.090 426	-0.236 426	-0.142 426	-0.105 426	0.191 426	-0.102 425	***n*** 426	0.225 426	0.066 426	0.019 426	0.174 425	0.174 426	-0.026 426	-0.077 426
Trait 9: HLC_0311::10023821927 GPX7 on Chr 1 @ 53.074723 Mb glutathione peroxidase 7	0.143 427	-0.312 427	-0.518 427	-0.660 427	-0.439 427	0.603 427	-0.109 426	0.214 426	***n*** 427	0.214 427	0.337 427	0.069 426	-0.007 427	-0.075 427	0.148 427

Trait															
Trait 10: HLC_0311::10025907159 GSR on Chr 8 @ 30.535579 Mb glutathione reductase	-0.296 427	0.268 427	0.077 427	-0.062 427	-0.272 427	0.100 427	0.037 426	0.004 426	0.252 427	***n*** 427	0.513 427	-0.204 426	-0.243 427	0.284 427	-0.077 427
Trait 11: HLC_0311::10025903928 GSS on Chr 20 @ 33.516235 Mb glutathione synthetase	-0.353 427	0.297 427	0.240 427	-0.071 427	-0.346 427	0.140 427	0.032 426	0.015 426	0.329 427	0.487 427	***n*** 427	-0.293 426	-0.354 427	0.177 427	0.126 427
Trait 12: HLC_0311::10023815327 SOD1 on Chr 21 @ 33.041243 Mb superoxide dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))	0.803 426	-0.512 426	-0.348 426	-0.514 426	0.283 426	0.220 426	-0.048 425	0.150 425	0.344 426	-0.124 426	-0.198 426	***n*** 426	0.962 426	-0.575 426	-0.224 426
Trait 13: HLC_0311::10033668826 SOD1 on Chr 21 @ 33.041243 Mb superoxide dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))	0.842 427	-0.492 427	-0.369 427	-0.524 427	0.324 427	0.152 427	-0.065 426	0.155 426	0.292 427	-0.161 427	-0.252 427	0.971 426	***n*** 427	-0.585 427	-0.254 427
Trait 14: HLC_0311::10023816501 SOD2 on Chr 6 @ 160.102754 Mb superoxide dismutase 2, mitochondrial	-0.696 427	0.372 427	0.249 427	0.327 427	-0.416 427	-0.092 427	0.099 426	-0.041 426	-0.119 427	0.264 427	0.120 427	-0.548 426	-0.554 427	***n*** 427	0.078 427

Trait 15: HLC_0311::10023810228 SOD3 on Chr 4 @ 24.802467 Mb superoxide dismutase 3, extracellular	-0.219 427	-0.091 427	0.018 427	0.133 427	-0.180 427	0.086 427	-0.038 426	-0.095 426	0.076 427	-0.080 427	0.113 427	-0.235 426	-0.254 427	0.021 427	***n*** 427