Article Title: Mechanisms of Antioxidant Induction with High-Dose N-acetylcysteine in Childhood Cerebral Adrenoleukodystrophy

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Supplementary Information

1. Methods

Determination of intracellular reactive oxygen species

For FACS analysis, cells seeded on 24-well plates were harvested and washed twice with PBS, and stained with 1 μM CM-H$_2$DCFDA for 5 minutes. The samples were subsequently washed twice and resuspended in 250μl PBS containing 5μl 7-AAD fluorescent probes for analysis. The percentage of positive stained CM-H$_2$DCFDA of live cells was used as the indicator for ROS levels in different treatment groups.

2. Supplementary Figures

Supplementary Fig 1: Heme oxygenase-1 expression in control versus childhood cerebral adrenoleukodystrophy fibroblasts following N-acetylcysteine exposure. Adrenoleukodystrophy fibroblasts showed significantly higher heme oxygenase-1 expression at baseline compared to
control fibroblasts. Exposure to increasing concentrations of N-acetylcysteine did not significantly alter heme oxygenase-1 levels in adrenoleukodystrophy fibroblasts. Data from representative experiment (n=3) is expressed as Mean ± SEM. **CCALD** Childhood cerebral adrenoleukodystrophy, **NAC** N-acetylcysteine, **HO-1** Heme oxygenase-1

**Supplementary Fig 2**: Changes in intracellular reactive oxygen species levels in control versus childhood cerebral adrenoleukodystrophy fibroblasts following N-acetylcysteine exposure. At baseline, childhood cerebral adrenoleukodystrophy fibroblasts have significantly higher (>15-fold) reactive oxygen species levels than control fibroblasts; two-way ANOVA *** p<0.001. Further, N-acetylcysteine exposure leads to concentration dependent increase in reactive oxygen species (up to 10-fold with highest N-acetylcysteine concentration) in control fibroblasts, while adrenoleukodystrophy fibroblasts showed ~1.5-fold increase in reactive oxygen species levels with increasing N-acetylcysteine exposure. Data from representative experiment (n=4) is expressed as Mean ± SEM. **ROS** reactive oxygen species; **CCALD** Childhood cerebral adrenoleukodystrophy; **NAC** N-acetylcysteine