MSD statistical analysis & volcano plots

This file provides an overview of linear mixed model analysis applied to MSD cytokine data in the HTP. It also provides the location and description of relevant volcano plots generated using the results of the analysis. associated with the analysis.

# Statistical Analysis

For each of 54 cytokines, five linear mixed models were fit to address different research questions. Each model can be thought of as running a t-test for each independent variable, with a shared budget of 0.05 for statistical significance. All p-values were corrected for multiple comparisons using the Benjamini and Hochberg correction for multiple comparisons. In corresponding volcano plots, corrected P-values are labeled as ‘FDR’, the False Discovery Rate.

## Overview of fitted models

**Model 1 (M1)**

* **Model specification\*:** log2(Concentration) ~ T21 + Age + Female sex + (1|Source)
* **Purpose:** Test for the effects of karyotype, age, and sex on average log2(concentration), after correction for batch effects related to sample source. Model 1 serves as the baseline model upon which models 2 and 3 are built.

**Model 2 (M2)**

* **Model specification\*:** log2(Concentration) ~ T21 + Age + Female sex + *T21\*Age* + (1|Source)
* **Purpose:** Test for the effects of karyotype, age, and sex on average log2(concentration), after correction for batch effects related to sample source. Test if the age-dependent trajectory of log2(concentration) differs by karyotype. This model is equivalent to Model 1, with the addition of T21\*Age.

**Model 3 (M3)**

* **Model specification\*:**  log2(Concentration) ~ T21 + Age + Female sex + *T21\*(Female sex)* + (1|Source)
* **Purpose:** Test for the effects of karyotype, age, and sex on average log2(concentration), after correction for batch effects related to sample source. Test if the effect of sex differs by karyotype. This model is equivalent to Model 1, with the addition of T21\*(Female sex).

**Model 1A (M1A)**

* **Model specification\*:** log2(Concentration) ~ Age + Female sex + (1|Source)
* **Purpose:** Test for the effects of age and sex in T21s only. This is a stratified version of Model 1.

**Model 1B (M1B)**

* **Model specification\*:** log2(Concentration) ~ Age + Female sex + (1|Source)
* **Purpose:** Test for the effects of age and sex in D21s only. This is a stratified version of Model 1.

## \*Model specifications are provided in syntax used by R lmerTest::lmer().

# Volcano plots

## General > Analyses > MSD\_T21\_D21\_comparison > Plots > Volcano

**Description:** Volcano plots for the effect of T21, as estimated from three linear mixed models with different sets of covariates. All plots in this folder use the same axis limits (‘matched scales’).

**Files:**

* MSD\_051220\_Volcano - Model 1 - Effect of T21 adjusted for age, sex, and sample source.pdf
* MSD\_051220\_Volcano - Model 1 - Effect of T21 adjusted for age, sex, and sample source.png
* MSD\_051220\_Volcano - Model 2 - Effect of T21 adjusted for age, sex, T21 by age, and sample source.pdf
* MSD\_051220\_Volcano - Model 2 - Effect of T21 adjusted for age, sex, T21 by age, and sample source.png
* MSD\_051220\_Volcano - Model 3 - Effect of T21 adjusted for age, sex, T21 by sex, and sample source.pdf
* MSD\_051220\_Volcano - Model 3 - Effect of T21 adjusted for age, sex, T21 by sex, and sample source.png

## General > Analyses > MSD\_Age\_comparison > Plots > Volcano > Matched scales

**Description:** Volcano plots for the effect of age overall, effect of age within T21s, effect of age within D21s, and effect of T21 on age-dependent trajectory trajectory (AKA differential effect of age by karyotype). All plots in this folder use the same axis limits (‘matched scales’).

**Files:**

* MSD\_051220\_Volcano\_MatchedScales - Model 1 - Effect of age overall.pdf
* MSD\_051220\_Volcano\_MatchedScales - Model 1 - Effect of age overall.png
* MSD\_051220\_Volcano\_MatchedScales - Model 1A - Effect of age in T21s.pdf
* MSD\_051220\_Volcano\_MatchedScales - Model 1A - Effect of age in T21s.png
* MSD\_051220\_Volcano\_MatchedScales - Model 1B - Effect of age in D21s.pdf
* MSD\_051220\_Volcano\_MatchedScales - Model 1B - Effect of age in D21s.png
* MSD\_051220\_Volcano\_MatchedScales - Model 2 - Effect of T21 on age-dependent trajectory.pdf
* MSD\_051220\_Volcano\_MatchedScales - Model 2 - Effect of T21 on age-dependent trajectory.png

## General > Analyses > MSD\_Age\_comparison > Plots > Volcano > Free scales

**Description:** Volcano plots for the effect of age overall, effect of age within T21s, effect of age within D21s, and effect of T21 on age-dependent trajectory (AKA differential effect of age by karyotype). For plots in this folder, axis limits vary based on the range of data plotted (‘free scales’).

**Files:**

* MSD\_051220\_Volcano\_FreeScales - Model 1 - Effect of age overall.pdf
* MSD\_051220\_Volcano\_FreeScales - Model 1 - Effect of age overall.png
* MSD\_051220\_Volcano\_FreeScales - Model 1A - Effect of age in T21s.pdf
* MSD\_051220\_Volcano\_FreeScales - Model 1A - Effect of age in T21s.png
* MSD\_051220\_Volcano\_FreeScales - Model 1B - Effect of age in D21s.pdf
* MSD\_051220\_Volcano\_FreeScales - Model 1B - Effect of age in D21s.png
* MSD\_051220\_Volcano\_FreeScales - Model 2 - Effect of T21 on age-dependent trajectory.pdf
* MSD\_051220\_Volcano\_FreeScales - Model 2 - Effect of T21 on age-dependent trajectory.png

## General > Analyses > MSD\_Sex\_comparison > Plots > Volcano > Matched scales

**Description:** Volcano plots for the effect of female sex overall, effect of female sex within T21s, effect of female sex within D21s, and differential effect of female sex by karyotype. All plots in this folder use the same axis limits (‘matched scales’).

**Files:**

* MSD\_051220\_Volcano\_MatchedScales - Model 1 - Effect of female sex overall.pdf
* MSD\_051220\_Volcano\_MatchedScales - Model 1 - Effect of female sex overall.png
* MSD\_051220\_Volcano\_MatchedScales - Model 1A - Effect of female sex in T21s.pdf
* MSD\_051220\_Volcano\_MatchedScales - Model 1A - Effect of female sex in T21s.png
* MSD\_051220\_Volcano\_MatchedScales - Model 1B - Effect of female sex in D21s.pdf
* MSD\_051220\_Volcano\_MatchedScales - Model 1B - Effect of female sex in D21s.png
* MSD\_051220\_Volcano\_MatchedScales - Model 3 - Differential effect of female sex by karyotype.pdf
* MSD\_051220\_Volcano\_MatchedScales - Model 3 - Differential effect of female sex by karyotype.png

## General > Analyses > MSD\_Sex\_comparison > Plots > Volcano > Free scales

**Description:** Volcano plots for the effect of female sex overall, effect of female sex within T21s, effect of female sex within D21s, and differential effect of female sex by karyotype. For each plot in this folder, axis limits vary based on the range of data plotted (‘free scales’).

**Files:**

* MSD\_051220\_Volcano\_FreeScales - Model 1 - Effect of female sex overall.pdf
* MSD\_051220\_Volcano\_FreeScales - Model 1 - Effect of female sex overall.png
* MSD\_051220\_Volcano\_FreeScales - Model 1A - Effect of female sex in T21s.pdf
* MSD\_051220\_Volcano\_FreeScales - Model 1A - Effect of female sex in T21s.png
* MSD\_051220\_Volcano\_FreeScales - Model 1B - Effect of female sex in D21s.pdf
* MSD\_051220\_Volcano\_FreeScales - Model 1B - Effect of female sex in D21s.png
* MSD\_051220\_Volcano\_FreeScales - Model 3 - Differential effect of female sex by karyotype.pdf
* MSD\_051220\_Volcano\_FreeScales - Model 3 - Differential effect of female sex by karyotype.png