Initial setup and analysis options

Several ‘pre-set’ analyses can be performed across these buttons

Click here to display the options for a custom analysis (see “Running analysis … “ slides)

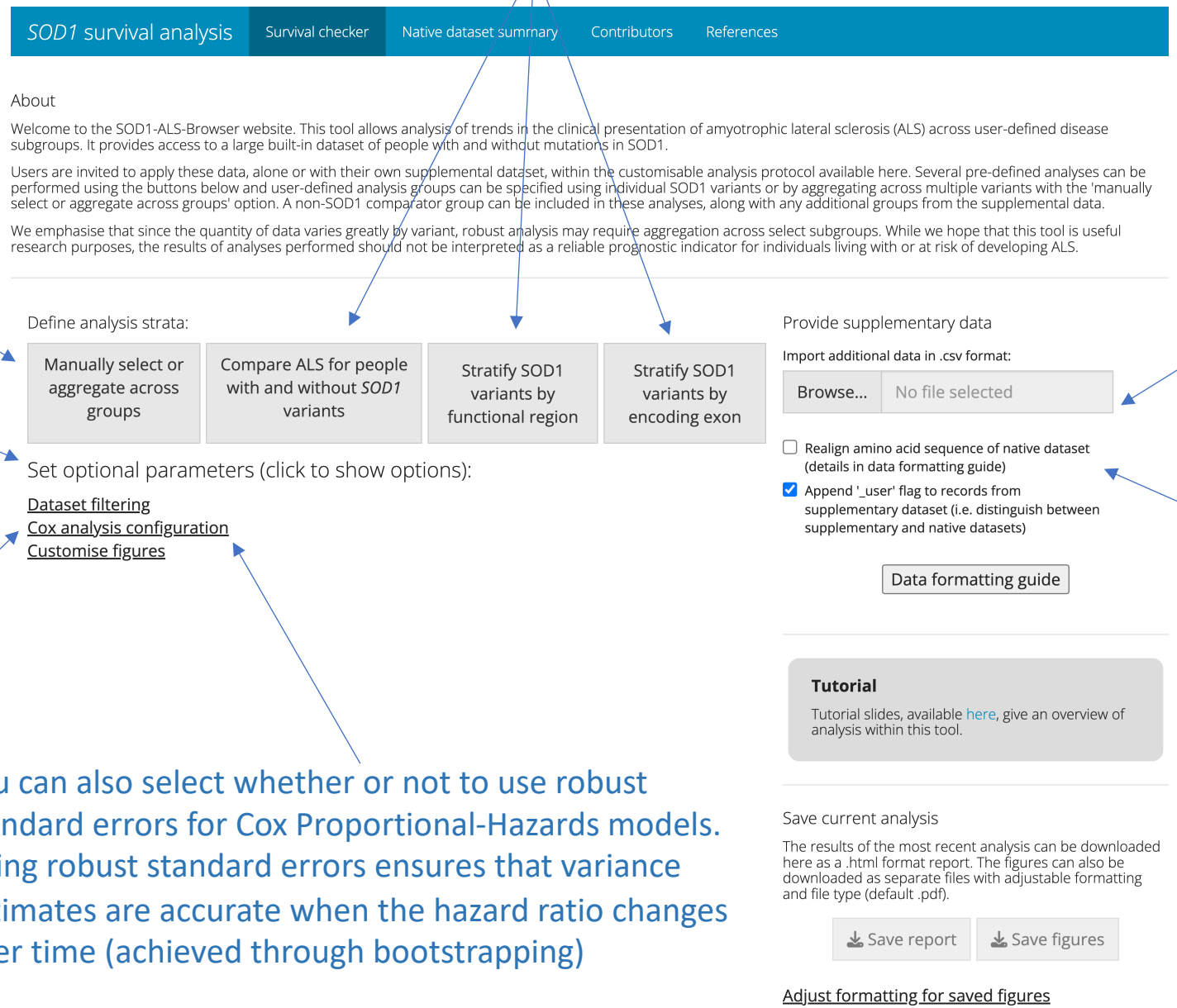
Various settings can be adjusted in this section. Click the buttons to see more!

Covariates used in multivariate analysis are adjusted here. Associations between analysis groups and available covariates can be tested once analysis groups

You can also select whether or not to use robust standard errors for Cox Proportional-Hazards models. Using robust standard errors ensures that variance estimates are accurate when the hazard ratio changes over time (achieved through bootstrapping)

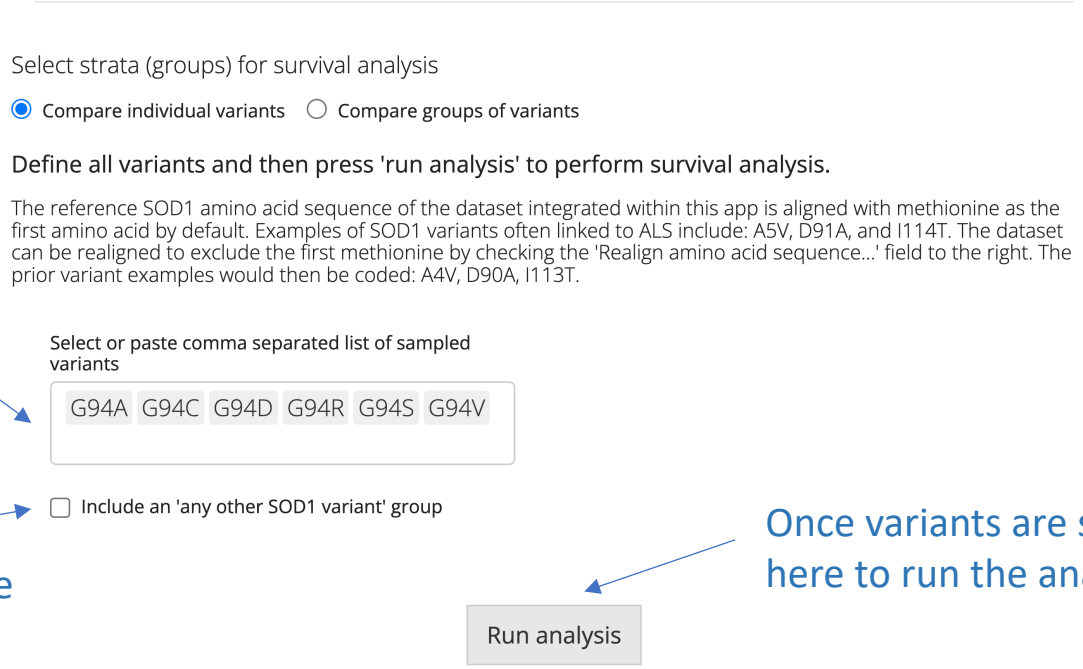
Supplementary data can be included within analyses, and should be uploaded here. Instructions for formatting are provided on the site

SOD1 sequence numbering can be toggled between the old and new nomenclature (including or excluding methionine at the start of the sequence)



Running analysis across individual variants

Use these buttons to choose between analysis across individual variants (this slide) or across variant groups (see next)



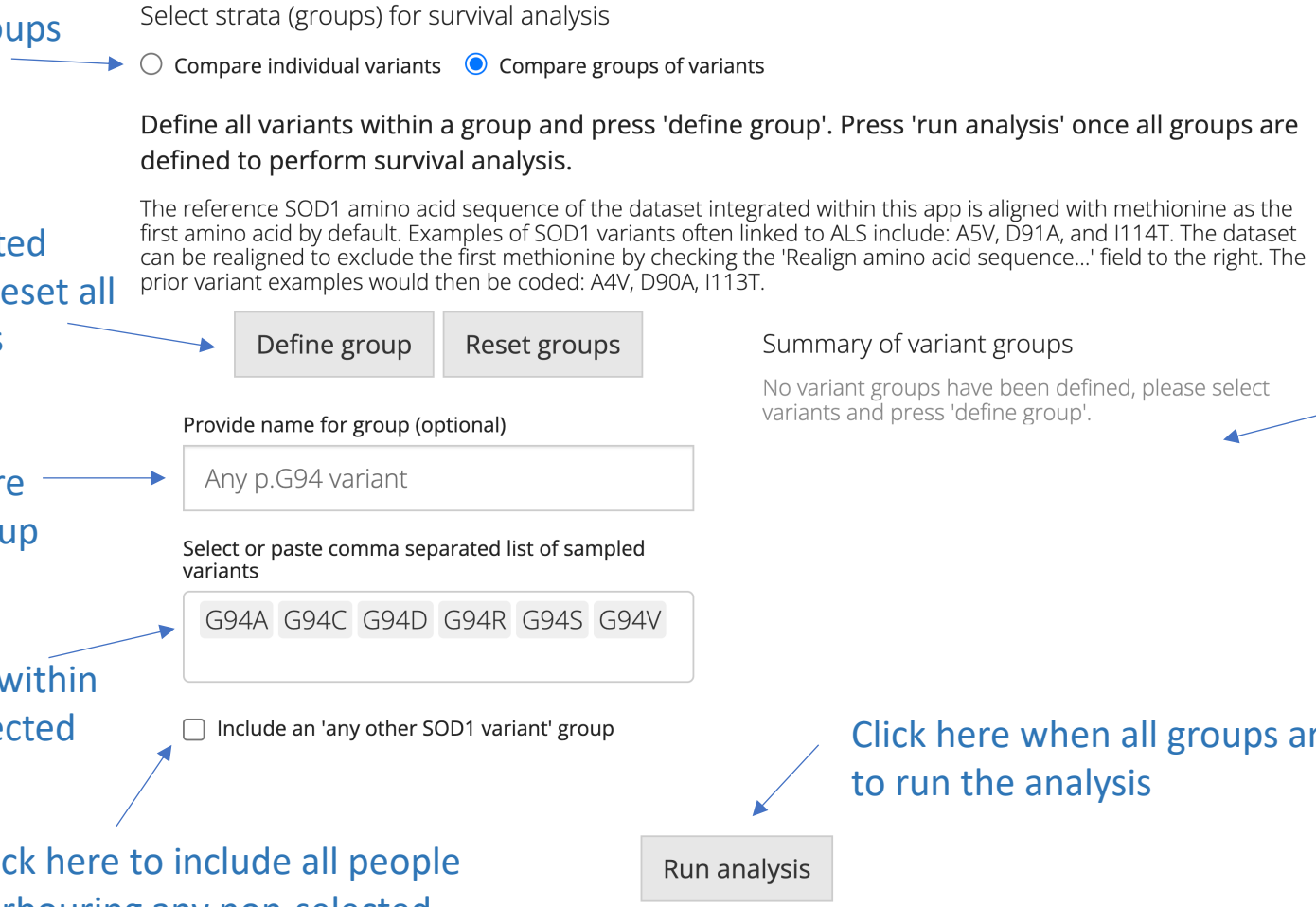
Variants available for analysis can be selected here

Click here to include all people harbouring any non-selected SOD1 variant within a single group

Once variants are selected, click here to run the analysis

Running analysis with groups

Use these buttons to choose between analysis across individual variants (previous slide) or across variant groups (this slide)



Declare the variants selected below as a new group or reset all groups with these buttons

Groups that have been already defined are summarised in a table shown here (see next slide for example)

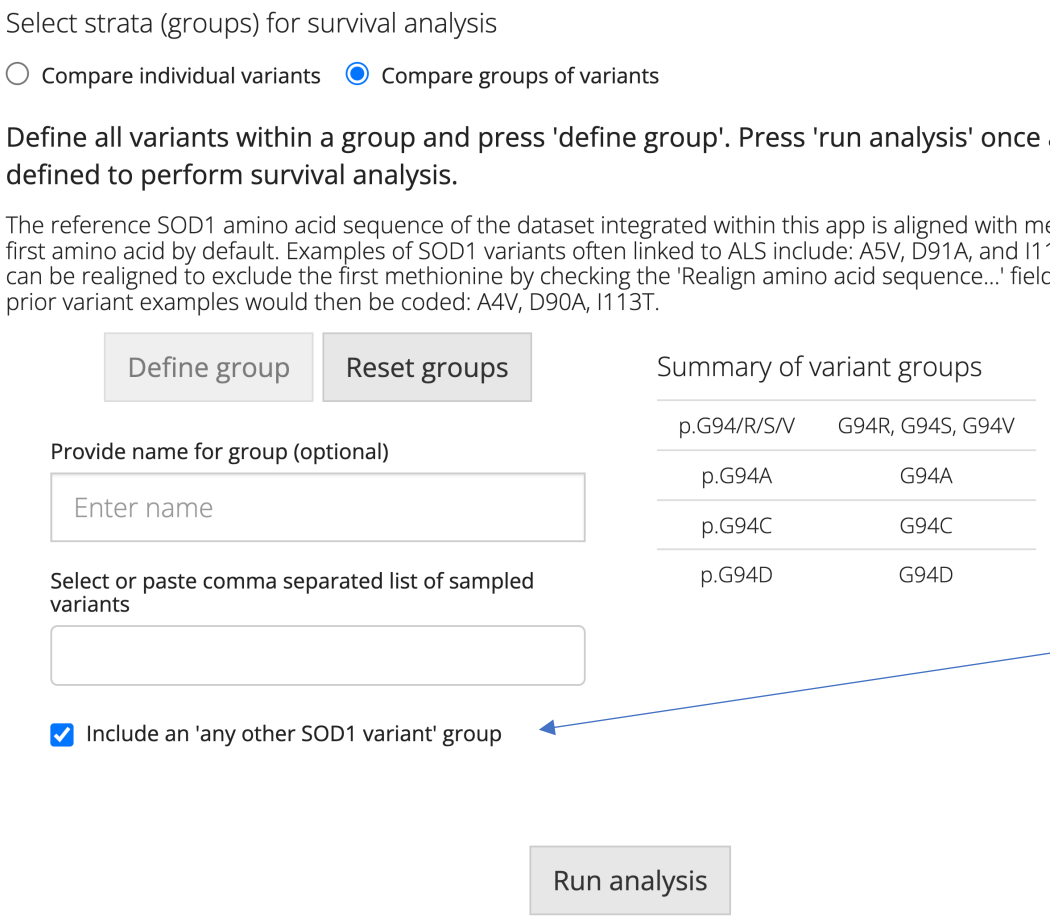
Groups can be named here (otherwise are called Group 1,..., N)

Variants to be included within a new group can be selected here

Click here to include all people harbouring any non-selected SOD1 variant within a single group

Click here when all groups are defined to run the analysis

Example analysis groups



These groups will be used for the subsequent analysis example

They will be compared additionally to the ‘OtherVariant’ group

Example analysis: data summaries

Each row of this table shows descriptive statistics for one of the analysis groups (strata). See below the table for more details.

				Age of onset in years			Disease duration in months			
Strata	Total sample size	Records with age of onset	Records with disease duration [N censored]	Quartiles (0% | 25% | median (50%) | 75% | 100%)	Mean [SD / SE]	Median estimate [95% CI]	Quartiles in people not-censored (0% | 25% | median (50%) | 75% | 100%)	Mean in people not-censored [SD]	Restricted mean estimate [SE]	Median estimate [95% CI]
OtherVariant	1320	1252	1034 [248]	13 | 41 | 49 | 57 | 85.52	49.07 [12.65 / 0.36]	49 [48, 50]	1 | 12 | 22.62 | 66 | 564.01	50 [64.7]	88.43 [4.83]	37.59 [30, 44]
p.G94A	27	27	26 [1]	19 | 37 | 48 | 61 | 78	48.7 [16.77 / 3.17]	48 [43, 61]	12 | 16 | 22 | 32 | 172	33.44 [33.42]	33.44 [6.55]	22 [19, 32]
p.G94C	14	14	9 [4]	31 | 34.25 | 37.5 | 44.88 | 65.98	40.46 [9.4 / 2.42]	37.5 [35, 51]	19.88 | 37.49 | 42.84 | 49.18 | 235.4	76.96 [89.24]	221.68 [86.97]	235.4 [42.84, NA]
p.G94D	15	15	14 [5]	17 | 40.5 | 51 | 58.98 | 74	49.73 [15.17 / 3.78]	51 [45, 63]	9 | 27.04 | 46 | 74.88 | 94	49.4 [30.13]	55.56 [8.64]	53 [31, NA]
p.G94R/S/V	7	7	3 [0]	30 | 35.5 | 39 | 44 | 46	39.14 [5.93 / 2.07]	39 [34, NA]	22 | 38.5 | 55 | 131 | 207	94.67 [98.67]	94.67 [46.51]	55 [22, NA]

Overview of analysis strata. Descriptive statistics are provided based on all raw data in age of onset analysis and people who are not censored in disease duration analysis. 'Estimated' values for median and mean are also shown based on the survival curve calculated by survfit, which takes into account any censoring in data. SE (standard error) and 95% CI (confidence intervals) pertain to estimated median/restricted means from the survival analysis. SD (standard deviation) and quartiles are associated with the raw data.

If your analysis includes strata which aggregate across subgroups, you can inspect boxplots in this section to understand the extent to which the survival curves differ across these subgroups.

Show median and restricted mean estimates for analysis of subgroups within analysis strata

Some quick comparisons between the strata and covariates available for use in Cox Proportional-Hazards models can be displayed by clicking here (the comparisons are shown in the tables/figures below)

View association between analysis strata and available Cox model covariates

Inferential statistics

	Test	Statistic	P-value
Diagnosis	Fisher's exact	-	0.2289
Family history	Fisher's exact	-	0.1064
Sex	Fisher's exact	-	0.01
Site of onset	Fisher's exact	-	0.8561
Region of origin	Fisher's exact	-	5e-04
Age of onset	ANOVA	F = 2.64	0.0325

Tests of difference between possible model covariates and strata of current survival analysis

Descriptive overview (select covariate)

Site of onset

You can choose which variable to inspect from this menu

	Spinal	Bulbar	Mixed	Respiratory
OtherVariant	972	107	8	8
p.G94A	25	1	0	0
p.G94C	8	0	0	0
p.G94D	15	0	0	0
p.G94R/S/V	6	0	0	0

Crosstabulation with survival analysis strata

Example analysis: univariate results

Analyses using survival analysis methodologies are shown across 2 columns. Age of symptom onset on the left, and disease duration (from onset until death or censoring) on the right

Kaplan-Meier plots display the survival curve for each analysis group

A significant log-rank test (p<0.5) suggests a difference in the median age of onset (or disease duration) across the analysis groups

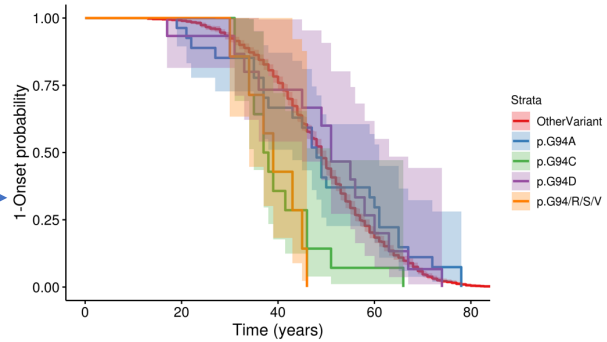
If testing 3+ strata, pairwise log-rank tests can be viewed by clicking here

Here are the hazard ratios from a Cox Proportional-Hazards model including no covariates (relative to the reference group – which has no estimate shown)

Age of onset analysis

Univariate analysis

Kaplan-Meier survival curve



Differences between strata

Global log-rank test: Chi-Squared = 23.7, p = 9.17e-05

Strata	Unadjusted Hazard Ratio [95% Confidence Interval]
OtherVariant	-
p.G94A	0.857 [0.561, 1.31]
p.G94C	2.4 [1.21, 4.76]
p.G94D	0.891 [0.57, 1.39]
p.G94/R/S/V	3.49 [2.27, 5.36]

Unadjusted hazard ratio for age of onset in analysis strata relative to the reference group, within Cox Proportional Hazards model excluding covariates.

Show pairwise log-rank results

Multivariate analysis

Cox proportional-hazards model

Model formula

```
Surv(`Age of onset`) ~ strata + Sex
```

Select reference category for strata variable in Cox model

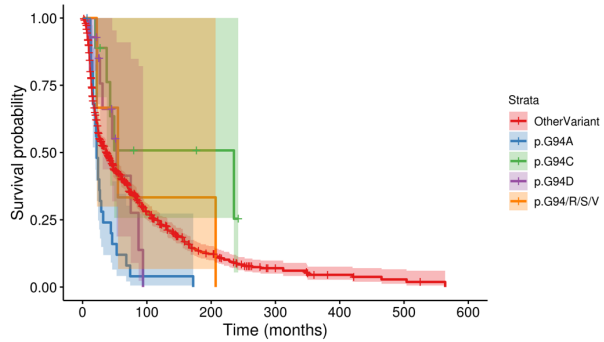
OtherVariant

Display advanced options for Cox model

Disease duration analysis

Univariate analysis

Kaplan-Meier survival curve



Differences between strata

Global log-rank test: Chi-Squared = 11.58, p = 0.0208

Strata	Unadjusted Hazard Ratio [95% Confidence Interval]
OtherVariant	-
p.G94A	1.75 [1.35, 2.28]
p.G94C	0.446 [0.197, 1.01]
p.G94D	0.89 [0.589, 1.35]
p.G94/R/S/V	0.85 [0.415, 1.74]

Unadjusted hazard ratio for disease duration in analysis strata relative to the reference group, within Cox Proportional Hazards model excluding covariates.

Show pairwise log-rank results

Multivariate analysis

Cox proportional-hazards model

Model formula

```
Surv(`Disease duration`, status) ~ strata + Sex + `Age of
```

Select reference category for strata variable in Cox model

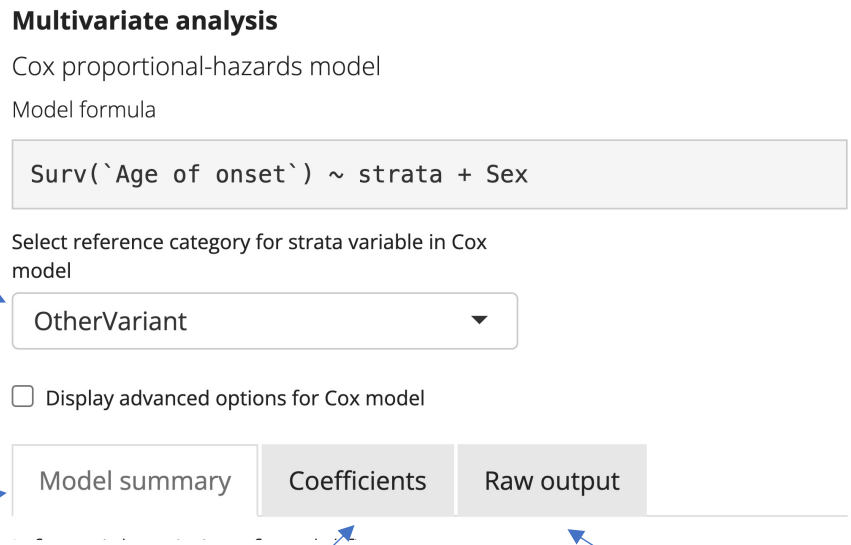
OtherVariant

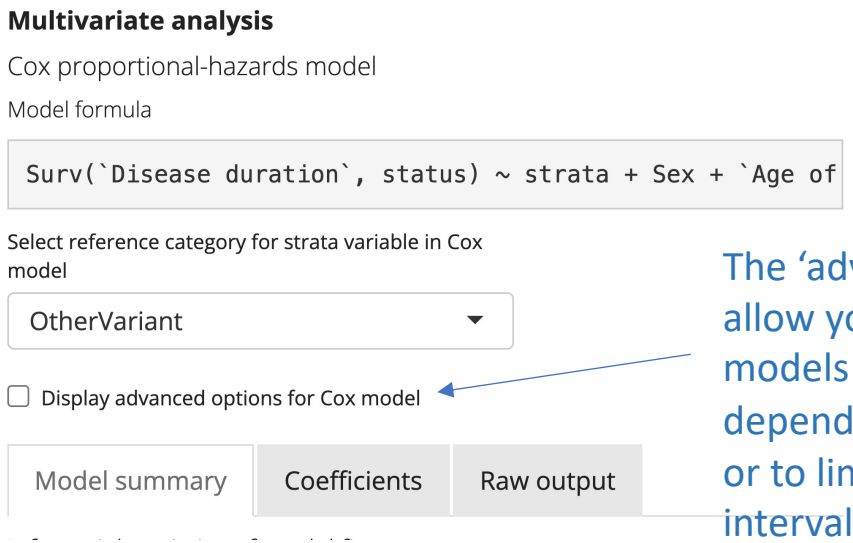
Display advanced options for Cox model

Example analysis: multivariate results (Cox Proportional-Hazards models)

Summaries of multivariate analyses, Cox Proportional-Hazards models, are next provided for analyses of age of onset (left) and disease duration (right)

You can select the reference group to use in the analyses here. The analysis will re-run which takes a few seconds





The 'advanced' options allow you to specify models with time-dependent coefficients or to limit the time-interval to consider in the survival analysis (e.g. only 12 months from symptom onset). More details on these options are within.

The 'model summary' tab contains general statistics about the fit of the model and returns the test of the proportional-hazards assumption

The 'coefficients' tab contains a table summarising all variables in the analysis, along with a forest plot visualising hazard ratios

This tab prints summaries directly from the R output for log-rank and Cox Proportional-Hazards analysis