TECHNICAL NOTE

Extending Olink's Mouse Exploratory panel to studies in rats

Animal models in translational research

Animal models have been used since the early beginnings of the biomedical sciences, and have played an important intermediate role in translating laboratory discoveries into eventual clinical application. This has been of particular importance in the pharmaceutical industry, where animal models have played key roles during pre-clinical drug development (e.g. in ADME/tox). Small mammals such as rodents have been extensively used as animal models for several reasons, including ease of maintenance, adaptability to new environments, short gestational cycle and well-characterized genetics and physiology.

There are of course natural dissimilarities between the pathophysiological mechanisms at play in different animal models and humans. This combined with ethical and economic considerations, has affected the use of animal research over time. In recent years, however, the use of small animal models such as those in mice has actually increased in translational medicine studies and drug development *(1)*. This is largely due to significant advances in molecular (principally genetic) technologies that enable the generation of human physiological system in animals. From the analysis perspective, however, the availability of rapid, high-multiplex tools that allow extended proteomic analysis in the limited sample volumes obtainable from such model systems has been extremely limited. It was for this reason that Olink developed and launched its Mouse Exploratory panel in 2018.

Mice have been particularly prevalent as small animal models due to the development of a well-established molecular toolbox that included the relatively early availability of mouse embryonic stem cells, providing germline access to the mouse and all of the possibilities for functional genomics that this opened up. The equivalent tools took many more years to be developed for rats, but this has since enabled a significant increase in their use as animal models. This is important because in some cases, the rat may provide a more relevant pathophysiological for humans than a mouse model, as been observed in studies of cardiovascular and neurological diseases and cancer *(2)*.

In this technical note, we will present data to show how Olink's Mouse Exploratory panel can be applied to studies using rat models.

Olink Mouse Exploratory panel

Olink® MOUSE EXPLORATORY was designed to meet our customers' need for protein biomarker studies in mice, where broad screening can facilitate the discovery of new markers and give new insights into biology.

The panel includes 92 proteins that are represented across 13 of our 14 human panels and is the highest multiplex protein assay currently on the market dedicated to mouse samples. To further meet the needs of our customers we have now conducted an investigation to determine how well this panel performs using rat samples from different rat stocks and strains, and different sample types.

Protein detection in rat samples

The proteins in the Mouse Exploratory panel have high homology between mouse and human (more than 80% in 93% of the markers) and also high homology between mouse and rat (more than 85% in 92% of the markers). This suggests that many of the assays in the Mouse Exploratory panel should also detect rat proteins.

Olink PEA method

A great advantage of the Proximity Extension Assay (PEA) method *(3)* is that a small volume of only 1 µl per panel is used for the analysis, which is particularly useful in rat and mouse models. It enables investigation of protein levels in several samples from different sample matrices at different time points, in the same rat. This can give more complete and continuous monitoring of individual rats. All samples in the following studies were analyzed using the Olink Mouse Exploratory panel.

Rat stocks and strains

In these studies, we investigated two of the most commonly used rat stocks, Wistar and Sprague Dawley. These are both outbred stocks with genetic variation within the stock. Both are multi-purpose stocks but there are some differences in phenotypes, where Wistar is more suitable for infectious disease models, and Sprague Dawley for studies on nutrition, diet-induced obesity or oncology. The third stock of rat included in this investigation is the Dahl strain which is most commonly used for studies of diet-induced hypertension.

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Outbred stocks have genetic variation within the stock. Genetic variation is maintained through well-defined rotational mating schemes, which are designed to maintain heterozygosity within the population over time.

Inbred strains have genetically identical individuals developed from brother and sister matings which have been carried out for twenty or more generations.

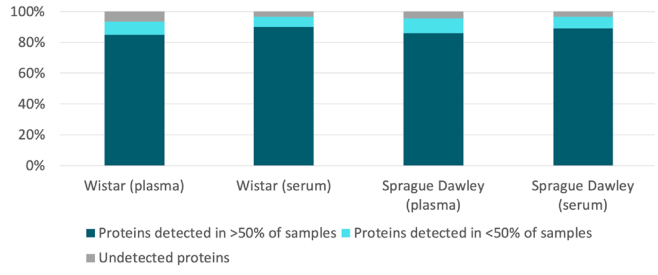
Detection in rat plasma and serum

Outbred stocks

We used samples from healthy, adult, female rats over 12 weeks of age from the Sprague Dawley and Wistar stocks. Plasma and matched serum samples from one rat from each stock were analyzed.

Results

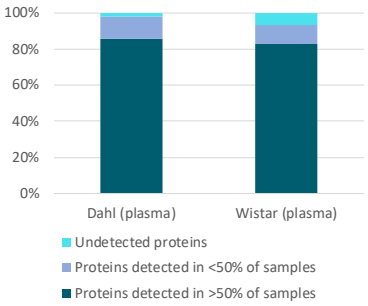
Samples (n)	Proteins detected in >50% of samples	Undetected proteins
Wistar, plasma (8)	78	6
Wistar, serum (4)	83	3
Sprague Dawley, plasma (8)	79	4
Sprague Dawley, serum (4)	82	3



Hypertension study

Plasma samples from treated and untreated inbred Dahl rats (n=24) from a hypertension model, as well as healthy Wistar female controls (n=6) were analyzed for protein detectability.

Samples	Proteins detected in >50% of samples	Undetected proteins
Plasma, hypertension model in Dahl rats, treated and untreated	79	2
Healthy Wistar females	76	6



The detectability in more than 50% of the samples was 86% in Dahl rats with induced hypertension and 83% in control Wistar rats.

TBI study

A pilot study conducted by Prof. Lars Hillered and Dr. Fredrik Clausen at the Department of Neuroscience at Uppsala University investigated the early events of Traumatic brain injury (TBI) using a rat model. TBI is a condition where the early stages cannot be investigated in humans since microdialysis (MD) samples cannot be collected until the patient is in the hospital, sometimes days after the accident occurs. Animal models are therefore needed for understanding the mechanisms behind TBI and detection of potential biomarkers for the early stages of this condition.

The animal experiments were approved by the Uppsala County Animal Ethics board and followed the rules and regulations of the Swedish Agricultural Board.

Five male, 10 weeks-old, Sprague Dawley rats were used, and three were subjected to central fluid percussion injury (cFPI) while two were sham-injured controls. One rat did not survive the cFPI procedure.

The microdialysis was performed using CMA-12 100 kDa cut-off probes with 4 mm PES membrane (CMA microdialysis, Stockholm, Sweden) with artificial cerebrospinal fluid (CSF) with added albumin in the

perfusate to optimize fluid recovery.

NOTE: *If a protein is too big to get through the membrane, it will not be detected in the samples.*

For more information about how the experiment was performed, contact support@olink.com.

MD samples

After cFPI or sham injury, two MD probes were stereotaxically inserted 4 mm into the cortex through the burr holes. MD sample collection commenced fifteen minutes after insertion. To study potential brain lateralization, samples were collected hourly from both the left and right brain hemispheres at six timepoints over six hours. The samples were stored at -20°C until analysis.

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Brain lateralization, asymmetry of the brain, is the tendency for some neural functions to be specialized to one brain hemisphere or the other. These fundamental differences in the processing of information between the hemispheres are found in many vertebrates.

Plasma samples

Blood samples were collected in EDTA treated test tubes at one and six hours after the start of MD collection. The blood samples were centrifuged and the plasma was collected and frozen. The samples were stored at -20°C until analysis.

CSF samples

CSF was extracted from the cisterna magna post mortem, at 6 hours after injury. The extraction was successful for three of the rats. The samples were stored at -20°C until analysis.

Statistical analysis

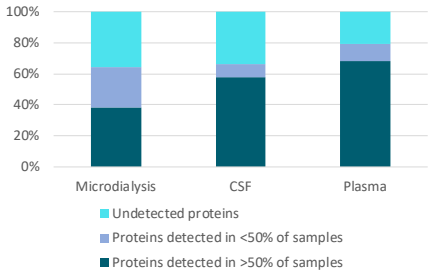
A linear mixed model was used to compare levels of proteins in MD samples from TBI-induced and sham rats over time. Only proteins detected in at least 50% of samples were included in the analysis, i.e. 35 proteins.

Results

Four Sprague Dawley rats, two with induced TBI and two sham, were analyzed.

Samples (n)	Proteins detected in >50% of samples	Undetected proteins
MD (8 samples, 6 time points)	35	33
CSF (3)	53	31
Plasma (4)	63	19

NOTE: *For MD samples, the maximum detectability out of the 6 time points is used as the detection percentage.*



The statistical analysis showed that:

- 10 of 35 proteins were significant for Time
- 1 of 35 proteins was significant for Condition (TBI/sham)

Detectability summary

The detectability of individual proteins in the Mouse Exploratory panel in the different groups and sample types is summarized in Table 1 for the Wistar and Dahl rats and in Table 2 for Sprague Dawley rats.

Summary for Wistar and Sprague Dawley

- Proteins detected in >50% of Sprague Dawley plasma samples ranges between 68-86%
- Proteins detected in >50% of Wistar plasma samples ranges between 78-85%
- 58 overlapping proteins detected in all plasma and serum, both stocks (63%)
- 58 proteins detected in all plasma and serum Sprague Dawley (63%)
- 67 proteins detected in all plasma and serum Wistar (73%)
- For matching plasma and serum samples, the detectability was consistent in individual mice for 83% of the assays in Wistar and 79% of the assays in Sprague Dawley.

If we combine results from all sample types, in all rats investigated in this paper (including Dahl rats) we have detection in more than 50% of samples in at least one sample type or group tested for 87 of 92 proteins (95%), and between 4-50% detection in at least one sample type or group for the remaining five proteins (see Tables 1 and 2).

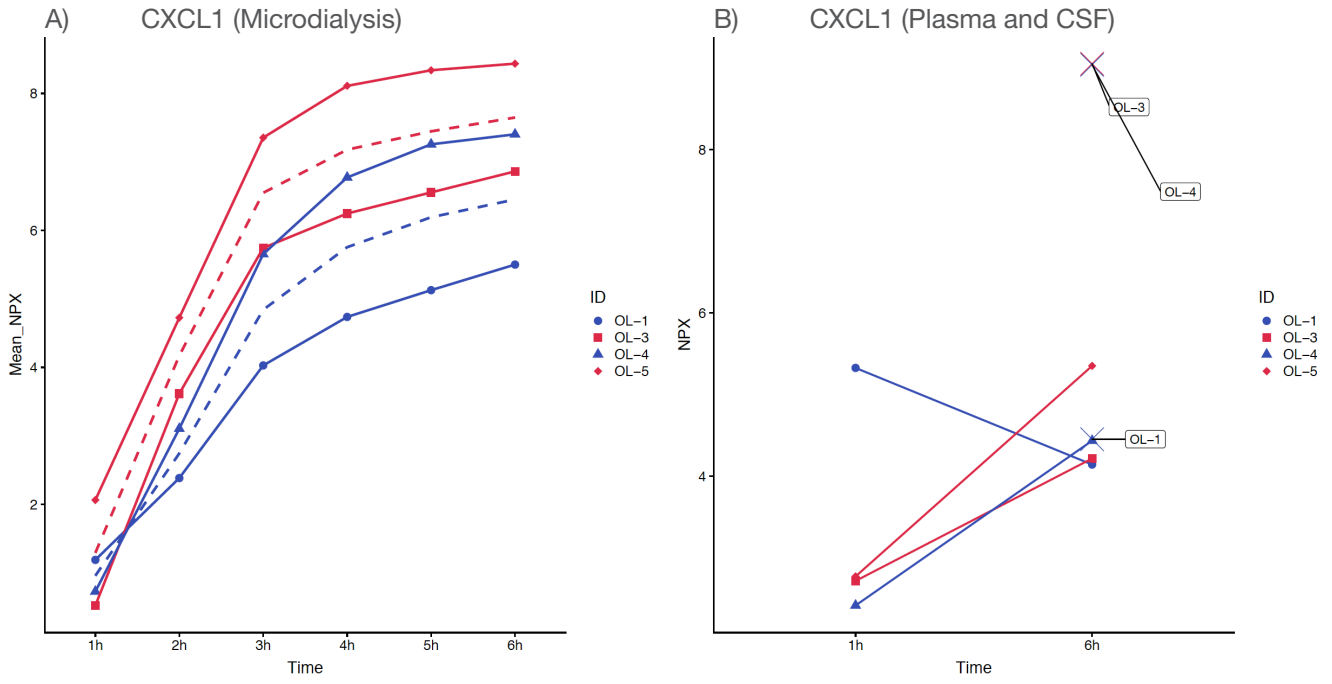


Figure 1 Detection of Cxcl1. Rats with induced TBI (OL-3 and OL-5) are marked in red and sham rats (OL-1 and OL-4) are marked in blue. A) Solid lines are the median of microdialysis values from both brain halves from the same rat. The dotted lines are the median of the two rats from the same group. B) Plasma samples from the same induced TBI and sham rats shown in panel A, taken at two time points. The Xs indicate single time point measurements of CXCL1 in CSF samples taken post-mortem from 3 of the 4 rats. The Xs show the values for CSF samples taken at 6 hours after injury. The plasma levels for CXCL1 increase over time, matching the increases in the brain.

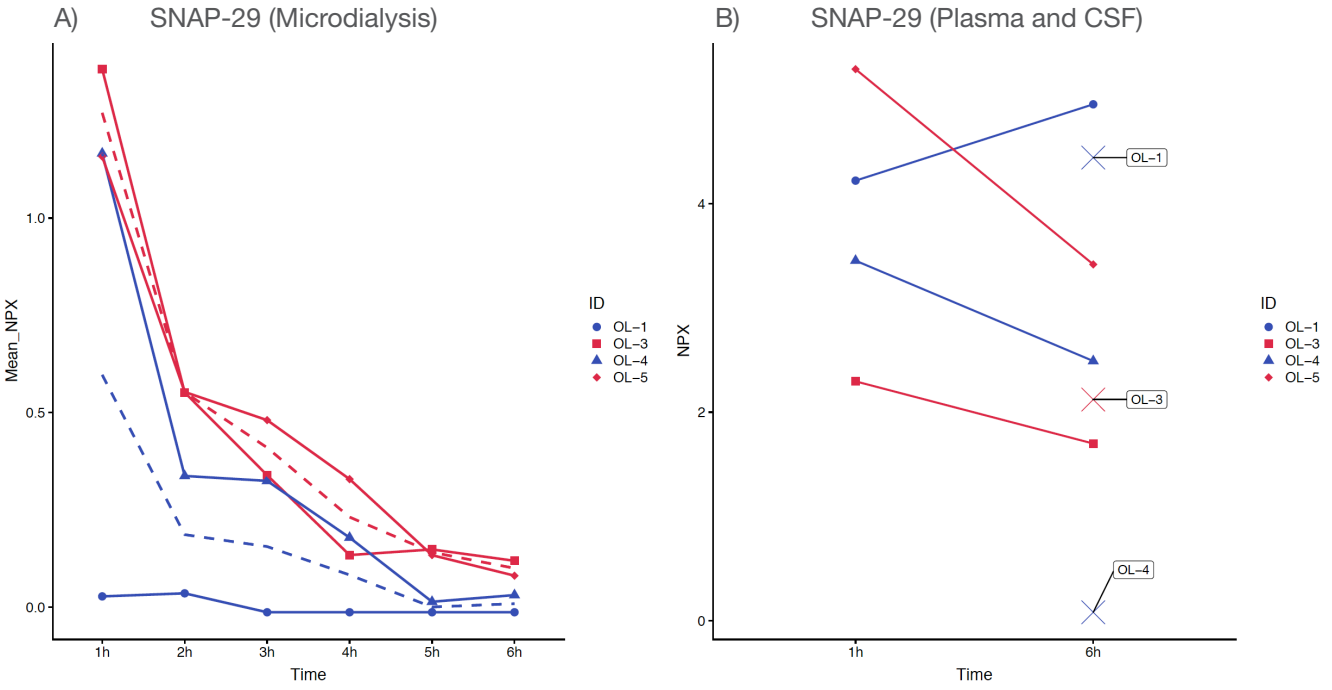


Figure 2 Detection of SNAP-29. Rats with induced TBI (OL-3 and OL-5) are marked in red and sham rats (OL-1 and OL-4) are marked in blue. A) Solid lines are the median of microdialysis values from both brain halves from the same rat. The dotted lines are the median of the two rats from the same group. B) The dots are plasma sample values and the lines indicate changes between time points for the same rat. The triangles show the values for CSF samples taken after 6 hours. MD levels of SNAP-29 are decreasing over time.

Cxcl1 and Snap-29

Looking more closely at some results for specific assays from the TBI study, we have chosen to present the protein levels in the MD samples from the brain as a whole, using an average of the samples from the two hemispheres.

In Figure 1 we can see the inflammatory marker, Growth-regulated alpha protein (CXCL1), showing a statistically significant increase over time. The increase is seen in both the rats with induced TBI as well as the sham, indicating that the insertion of the probe in itself triggers an inflammatory response.

Table 1 Protein detection percentage for Wistar and Dahl rats. Assay detection in eight plasma and four matched serum samples from Wistar rats and plasma from healthy female Wistar rats used as controls and plasma from Dahl rats with hypertension from the hypertension study.

Uniprot ID	Gene name	Wistar plasma (n=8)	Wistar serum (n=4)	Wistar plasma controls (n=5)	Dahl plasma (n=24)
Q61288	**Acvrl1**	88%	75%	33%	25%
Q9R1V7	**Adam23**	100%	100%	100%	100%
P30561	**Ahr**	13%	50%	0%	38%
Q8R5A3	**Apbb1ip**	0%	25%	67%	67%
O35625	**Axin1**	100%	100%	100%	100%
Q9D6N1	**Ca13**	75%	75%	17%	21%
Q8VCF1	**Cant1**	100%	100%	100%	100%
P70677	**Casp3**	100%	100%	100%	100%
P10148	**Ccl2**	100%	100%	100%	100%
O89093	**Ccl20**	100%	100%	83%	97%
P10855	**Ccl3**	100%	100%	100%	100%
P30882	**Ccl5**	100%	100%	100%	100%
P97326	**Cdh6**	100%	100%	100%	100%
Q8R373	**Clmp**	100%	100%	100%	100%
Q9ER65	**Clstn2**	13%	75%	17%	13%
P12960	**Cntn1**	100%	100%	100%	100%
Q69Z26	**Cntn4**	100%	100%	100%	100%
Q00493	**Cpe**	100%	100%	100%	100%
Q9JLL0	**Crim1**	100%	100%	100%	100%
P01587	**Csf2**	0%	50%	0%	0%
P12850	**Cxcl1**	100%	100%	100%	100%
P18340	**Cxcl9**	100%	100%	100%	100%
P18406	**Cyr61**	100%	100%	100%	100%
Q99KJ8	**Dctn2**	88%	100%	100%	100%
Q9CWS0	**Ddah1**	100%	100%	100%	100%
Q09163	**Dlk1**	100%	100%	100%	100%
Q61483	**Dll1**	100%	100%	100%	100%
Q8BX35	**Eda2r**	100%	100%	100%	100%
P17183	**Eno2**	100%	100%	100%	100%
Q99JW5	**Epcam**	100%	100%	100%	100%
P07321	**Epo**	100%	100%	100%	100%
Q61527	**Erbb4**	100%	100%	100%	100%
P25446	**Fas**	0%	0%	0%	0%
P26323	**Fli1**	38%	0%	33%	83%
Q8BLU0	**Flrt2**	100%	100%	100%	100%
Q9R1E0	**Foxo1**	75%	75%	50%	83%
P47931	**Fst**	100%	100%	100%	100%
Q9EQC7	**Fstl3**	100%	100%	100%	100%
P55095	**Gcg**	100%	100%	100%	100%
P48540	**Gdnf**	100%	100%	100%	100%
P97785	**Gfra1**	100%	100%	100%	88%
Q9EQX0	**Ghrl**	100%	100%	100%	100%
Q08048	**Hgf**	100%	100%	100%	100%
Q6ZQA6	**Igsf3**	100%	100%	100%	100%
P18893	**Il10**	38%	75%	33%	75%
Q62386	**Il17a**	100%	100%	100%	100%
Q7TNI7	**Il17f**	88%	100%	0%	38%
P01582	**Il1a**	13%	75%	0%	29%
P10749	**Il1b**	25%	50%	0%	42%
Q8K4B4	**Il23r**	0%	25%	17%	13%
P04401	**Il5**	0%	0%	0%	4%
P08505	**Il6**	25%	75%	100%	96%
Q9R000	**Itgb1bp2**	88%	100%	100%	100%
Q9Z0T9	**Itgb6**	100%	100%	67%	71%
P20826	**Kitlg**	75%	75%	100%	100%
O89017	**Lgmn**	100%	100%	100%	100%
P11152	**Lpl**	100%	100%	100%	100%
P70236	**Map2k6**	100%	100%	100%	100%
O08746	**Matn2**	100%	100%	100%	100%
Q61865	**Mia**	100%	100%	100%	100%
P58058	**Nadk**	100%	100%	100%	100%
Q61982	**Notch3**	100%	100%	100%	100%
P20181	**Ntf3**	100%	100%	100%	100%
Q8BTW9	**Pak4**	63%	100%	83%	92%
P11103	**Parp1**	100%	100%	100%	100%
P31240	**Pdgfb**	100%	100%	100%	100%
P47713	**Pla2g4a**	100%	100%	100%	100%
Q8CGN5	**Plin1**	38%	25%	33%	38%
Q80UG2	**Plxna4**	100%	100%	100%	100%
Q9DCL8	**Ppp1r2**	63%	75%	67%	75%
P99029	**Prdx5**	100%	100%	100%	100%
Q8BVI4	**Qdpr**	100%	100%	100%	100%
Q6PCX7	**Rgma**	100%	100%	100%	100%
Q8CD15	**Riox2**	100%	100%	100%	100%
P07091	**S100a4**	100%	100%	100%	100%
Q4V9Z5	**Sez6l2**	100%	100%	100%	100%
Q9ERB0	**Snap29**	100%	100%	100%	100%
P48030	**Tgfa**	100%	100%	100%	100%
P04202	**Tgfb1**	100%	100%	100%	100%
O88393	**Tgfbr3**	100%	100%	100%	100%
P06804	**Tnf**	0%	75%	17%	25%
O08712	**Tnfrsf11b**	100%	100%	100%	100%
Q9CR75	**Tnfrsf12a**	100%	100%	100%	100%
O54907	**Tnfsf12**	100%	100%	100%	100%
P48787	**Tnni3**	100%	100%	100%	100%
Q8BYI9	**Tnr**	100%	100%	100%	100%
O89023	**Tpp1**	100%	100%	100%	100%
P97946	**Vegfd**	100%	100%	100%	100%
Q9Z109	**Vsig2**	100%	100%	100%	100%
Q7TQN3	**Wfikkn2**	100%	100%	100%	100%
O54775	**Wisp1**	100%	100%	100%	100%
Q04736	**Yes1**	100%	100%	100%	100%

Although there are few rats used in this pilot study there is a trend towards higher levels in the TBI-induced rats from three hours and onward when comparing the averages between this group and the sham.

An increase in CXCL1 in the plasma samples between 1 and 6 hours is seen in three of four animals. This could possibly reflect the inflammatory response seen in the MD samples in these rats. The decrease in plasma levels of CXCL1 rat OL-4 (the sham rat with the lowest levels of CXCL1 in the MD samples) indicates that plasma is not as good as MD samples to measure changes in the brain. In CSF there are also very high levels of CXCL1 in two rats corresponding to the high levels in their MD samples, while the rat with the lowest levels in the MD samples has the same levels in CSF as in plasma.

Thus, for this disease model the most useful sample type to follow the events following the injury is MD fluid, although CSF and plasma might also reflect these events to some extent.

Table 2 Protein detection percentage for Sprague Dawley rats. Assay detection in eight plasma and four matched serum samples from Sprague Dawley (SD) rats as well as samples from the TBI-study. For the TBI study the results for plasma are presented separately for sham and TBI induced groups, for other matrices results are presented as one group.

Uniprot ID	Gene name	SD plasma (n=8)	SD serum (n=4)	SD plasma TBI sham (n=2)	SD plasma TBI ind. (n=2)	SD CSF TBI study (n=3)	SD MD TBI study (n=4)
Q61288	**Acvrl1**	100%	100%	0%	0%	33%	100%
Q9R1V7	**Adam23**	100%	100%	100%	100%	0%	0%
P30561	**Ahr**	38%	0%	0%	0%	0%	0%
Q8R5A3	**Apbb1ip**	0%	25%	0%	0%	0%	0%
O35625	**Axin1**	100%	100%	100%	50%	67%	0%
Q9D6N1	**Ca13**	88%	100%	0%	50%	33%	25%
Q8VCF1	**Cant1**	88%	100%	0%	50%	0%	0%
P70677	**Casp3**	100%	100%	100%	100%	100%	38%
P10148	**Ccl2**	100%	100%	100%	100%	100%	100%
O89093	**Ccl20**	13%	50%	0%	0%	67%	0%
P10855	**Ccl3**	100%	100%	100%	100%	100%	100%
P30882	**Ccl5**	100%	100%	100%	100%	100%	50%
P97326	**Cdh6**	100%	100%	100%	100%	67%	0%
Q8R373	**Clmp**	100%	100%	100%	100%	100%	50%
Q9ER65	**Clstn2**	38%	100%	50%	0%	100%	38%
P12960	**Cntn1**	100%	100%	100%	100%	100%	75%
Q69Z26	**Cntn4**	100%	100%	100%	100%	100%	25%
Q00493	**Cpe**	100%	100%	100%	100%	100%	88%
Q9JLL0	**Crim1**	100%	100%	100%	100%	67%	25%
P01587	**Csf2**	13%	75%	0%	0%	0%	0%
P12850	**Cxcl1**	100%	100%	100%	100%	100%	100%
P18340	**Cxcl9**	100%	100%	100%	100%	100%	13%
P18406	**Cyr61**	100%	100%	100%	100%	100%	38%
Q99KJ8	**Dctn2**	88%	100%	0%	100%	100%	13%
Q9CWS0	**Ddah1**	100%	100%	50%	100%	100%	75%
Q09163	**Dlk1**	100%	100%	100%	100%	100%	100%
Q61483	**Dll1**	100%	100%	100%	100%	0%	0%
Q8BX35	**Eda2r**	100%	100%	100%	100%	100%	100%
P17183	**Eno2**	100%	100%	100%	100%	100%	88%
Q99JW5	**Epcam**	100%	100%	100%	100%	0%	100%
P07321	**Epo**	100%	100%	100%	100%	0%	0%
Q61527	**Erbb4**	100%	100%	100%	100%	100%	100%
P25446	**Fas**	25%	25%	0%	0%	0%	0%
P26323	**Fli1**	38%	50%	0%	0%	0%	0%
Q8BLU0	**Flrt2**	100%	100%	100%	100%	100%	38%
Q9R1E0	**Foxo1**	100%	100%	50%	0%	33%	0%
P47931	**Fst**	100%	100%	100%	100%	100%	50%
Q9EQC7	**Fstl3**	100%	100%	100%	100%	100%	100%
P55095	**Gcg**	100%	100%	100%	100%	0%	0%
P48540	**Gdnf**	100%	100%	0%	100%	0%	0%
P97785	**Gfra1**	100%	100%	100%	100%	100%	100%
Q9EQX0	**Ghrl**	100%	100%	100%	100%	0%	0%
Q08048	**Hgf**	100%	100%	100%	100%	100%	38%
Q6ZQA6	**Igsf3**	100%	100%	100%	100%	100%	0%
P18893	**Il10**	38%	75%	0%	0%	0%	0%
Q62386	**Il17a**	100%	100%	50%	50%	0%	0%
Q7TNI7	**Il17f**	100%	100%	0%	0%	0%	0%
P01582	**Il1a**	0%	75%	0%	0%	67%	25%
P10749	**Il1b**	63%	75%	0%	0%	0%	13%
Q8K4B4	**Il23r**	0%	25%	0%	0%	0%	0%
P04401	**Il5**	0%	0%	0%	0%	0%	0%
P08505	**Il6**	75%	50%	0%	0%	100%	88%
Q9R000	**Itgb1bp2**	75%	50%	0%	0%	0%	0%
Q9Z0T9	**Itgb6**	100%	100%	100%	0%	0%	88%
P20826	**Kitlg**	75%	100%	0%	0%	0%	100%
O89017	**Lgmn**	100%	100%	100%	100%	100%	100%
P11152	**Lpl**	100%	100%	100%	100%	33%	13%
P70236	**Map2k6**	100%	100%	100%	100%	67%	63%
O08746	**Matn2**	100%	100%	100%	100%	100%	0%
Q61865	**Mia**	100%	100%	100%	100%	100%	0%
P58058	**Nadk**	100%	100%	100%	100%	100%	50%
Q61982	**Notch3**	100%	100%	100%	100%	0%	0%
P20181	**Ntf3**	100%	100%	100%	100%	0%	0%
Q8BTW9	**Pak4**	88%	100%	0%	0%	0%	0%
P11103	**Parp1**	100%	100%	100%	100%	67%	50%
P31240	**Pdgfb**	100%	100%	100%	100%	67%	25%
P47713	**Pla2g4a**	100%	100%	100%	100%	67%	0%
Q8CGN5	**Plin1**	13%	0%	0%	0%	0%	0%
Q80UG2	**Plxna4**	100%	100%	50%	50%	33%	0%
Q9DCL8	**Ppp1r2**	63%	75%	0%	0%	33%	0%
P99029	**Prdx5**	100%	100%	100%	100%	100%	88%
Q8BVI4	**Qdpr**	100%	100%	100%	100%	100%	100%
Q6PCX7	**Rgma**	100%	100%	100%	100%	100%	100%
Q8CD15	**Riox2**	100%	100%	100%	100%	100%	13%
P07091	**S100a4**	100%	100%	100%	100%	67%	13%
Q4V9Z5	**Sez6l2**	100%	100%	100%	100%	100%	63%
Q9ERB0	**Snap29**	100%	100%	100%	100%	100%	88%
P48030	**Tgfa**	100%	100%	100%	100%	100%	100%
P04202	**Tgfb1**	100%	100%	100%	100%	100%	25%
O88393	**Tgfbr3**	100%	100%	100%	100%	100%	100%
P06804	**Tnf**	25%	75%	50%	0%	0%	13%
O08712	**Tnfrsf11b**	100%	100%	100%	50%	0%	100%
Q9CR75	**Tnfrsf12a**	100%	100%	100%	100%	100%	38%
O54907	**Tnfsf12**	100%	100%	100%	100%	100%	13%
P48787	**Tnni3**	100%	100%	50%	100%	33%	0%
Q8BYI9	**Tnr**	100%	100%	100%	100%	100%	75%
O89023	**Tpp1**	100%	100%	100%	100%	0%	13%
P97946	**Vegfd**	100%	100%	100%	100%	0%	100%
Q9Z109	**Vsig2**	100%	100%	100%	100%	0%	38%
Q7TQN3	**Wfikkn2**	100%	100%	100%	100%	100%	100%
O54775	**Wisp1**	100%	100%	100%	100%	100%	25%
Q04736	**Yes1**	100%	100%	100%	50%	33%	13%

In the current investigation we could also detect proteins with statistically significant decreases over time, such as Synaptosomal-associated protein 29 (SNAP-29) as shown in Figure 2. This protein also shows a trend towards differences between TBI and sham at the earlier time points after the injury.

Discussion

Detectability differences between and within rat stocks

For the Sprague Dawley rats there were two groups that differed in sex and age: the matched serum/plasma samples from a normal female rat, and the younger male rats included in the TBI study. Detectability in >50% of

the plasma samples for these groups was 86% and 68% respectively.

For Wistar rats, a comparison was made between the plasma samples from the >12 weeks-old female rats, and the female controls used in the hypertension study. Here the detectability in >50% of the samples was 85% and 83% respectively.

In the hypertension study, we compared Dahl rats and Wistar rats. The genetic similarity between outbred Wistar rats and inbred Dahl rats has been reported to be approximately 50% by St Lezin et al. *(4)*.

In summary, we find differences in detectability of proteins between different rat stocks, between groups within the same stock, as well as between sexes. This can be expected considering the genetic diversity in the outbred stocks where individual protein levels can differ. In addition, different conditions and disease models have an effect on the protein levels and can affect the detectability of proteins that are normally at low levels.

Tumor necrosis factor receptor superfamily member 6 (FAS), Granulocyte-macrophage colony-stimulating factor (GM-CSF) and Interleukin-5 (IL-5) had low levels of detection in the investigated samples. This is consistent with results in healthy mice, where both GM-CSF and IL-5 have lower detection in serum and plasma samples but these proteins can be detected at higher levels in some diseases, for example during sepsis for IL-5.

As described in detail in the results section, the inflammatory marker CXCL1 increases over time in MD samples following an invasive surgical procedure and a reflection of this increase might also be seen to some extent in plasma and CSF (Figure 1). Similar increases in protein levels of IL-6, CCL2 and CCL3 in MD samples were observed in this investigation and such increases in inflammatory cytokines have been reported previously in the literature.

Kumar et al. showed that mRNA levels of both CCL2 and CCL3 were statistically increased in C57Bl/6 mice with TBI injury compared to sham at 24 hours after the injury *(5)*. Given the similar trends we saw for these markers in our studies, this suggests that these inflammatory responses following TBI can be seen at both the protein and mRNA level.

In a study by Rowe et al. from 2016 *(6)*, a significant protein increase in CXCL1 (at 1 hour and 3 hours) and Il-6 (at 1 hour) after injury was seen in C57Bl/6 mice. In this case, the measurements were taken in protein lysates from the cortex of affected animals. Our current study and these previous reports, shows a consistent picture where an increase in inflammatory cytokines can be seen after injury in a range of rat strains and stocks, and in different sample types.

The low volume requirements enabled multiple time points to be measured in MD samples taken from the same animal using the Olink panel, providing a more detailed picture. At the same time, a large number of proteins could be measured in each sample.

SNAP-29, which showed a decrease over time in MD samples from the rats in the TBI investigation (Figure 2) has not been previously associated with TBI. Reportedly, it is expressed in the pre-synapse where it binds to syntaxin and modulates the pre-synaptic machinery involved in vesicle membrane function. This study suggests that SNAP-29 could be a potentially interesting protein marker for TBI that can be investigated further.

In conclusion, this investigation shows that the Olink Mouse Exploratory panel can be used for measurement of protein levels in rat to confirm earlier findings as well as for identifying interesting new markers.

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Olink Proteomics
Dag Hammarskjölds v. 52B
SE-752 37 Uppsala, Sweden
www.olink.com