Prediction of Stroke Outcome in Mice Based on Non-Invasive MRI and Behavioral Testing

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ABSTRACT

Background: Prediction of post-stroke outcome using the degree of subacute deficit or magnetic resonance imaging is well studied in humans. While mice are the most commonly used animals in preclinical stroke research, systematic analysis of outcome predictors is lacking.

Methods: Data from 13 studies that included 45 min of middle cerebral artery occlusion on 148 mice were pooled. Motor function was measured using a modified protocol for the staircase test of skilled reaching. Phases of subacute and residual deficit were defined. Magnetic resonance images of stroke lesions were co-registered on the Allen Mouse Brain Atlas to characterize stroke topology. Different random forest prediction models that either used motor-functional deficit or imaging parameters were generated for the subacute and residual deficits.

Results: We detected both a subacute and residual motor-functional deficit after stroke in mice. Different functional severity grades and recovery trajectories could be observed. In mice with small cortical lesions, lesion volume was the best predictor of the subacute deficit. The residual deficit could be predicted most accurately by the degree of the subacute deficit. When using imaging parameters for the prediction of the residual deficit, including information about the lesion topology increased prediction accuracy. A subset of anatomical regions within the ischemic lesion had particular impact on the prediction of long-term outcome. Prediction accuracy depended on the degree of functional impairment.

Conclusions: For the first time, we identified and characterized predictors of post-stroke outcome in a large cohort of mice and found strong concordance with clinical data. These results are discussed in light of study design and imaging limitations. In the future, using outcome prediction can improve the design of preclinical studies and guide intervention decisions.

NON-STANDARD ABBREVIATIONS AND ACRONYMS

AMBA Allen Mouse Brain Atlas
CCA Common Carotid Artery
ECA External Carotid Artery
FOV Field Of View
ICA Internal Carotid Artery
IQR Interquartile Range
LSMD Least Square Mean Difference
MD Mean difference
MCA Middle Cerebral Artery
MCAO Middle Cerebral Artery Occlusion
MedAE Median Absolute Error
PA Pterygopalatine Artery
pp percentage points
PE prediction error

Keywords
stroke, mice, outcome prediction, motor-function, staircase

INTRODUCTION

Stroke remains a major public health challenge, as its occurrence rises in aging societies.[1] With decreasing mortality rates due to progress in acute stroke therapy, the need to develop treatments that improve functional outcome has become more pressing.[2] Understanding predictors of functional outcome after stroke both in humans and rodents is an essential step towards defining adequate treatment decisions and improving the design of preclinical studies.[3] Prediction of expected motor-functional outcomes in mice could potentially guide researchers in their treatment decisions in preclinical stroke intervention studies and support outcome-dependent stratifications. In humans, magnetic resonance imaging (MRI) is used to assess stroke volume and topology.[4] Lesion volume is most commonly used to analyze stroke severity and predict functional outcome, but the prognostic value has been shown to be better when including lesion topology.[5–11] This was also shown in an exploratory preclinical study using a porcine stroke model.[12] Additionally, the degree of the subacute deficit has been shown to be a reliable predictor of long-term outcome, especially for mild functional deficits.[13,14] In contrast to humans, little is known about predictors of functional outcome after stroke in mice, although they are commonly used in preclinical research. The stroke preclinical assessment network, for instance, recently completed the largest preclinical stroke study on mice and provided examples of how the middle cerebral artery occlusion (MCAO) model can be used to conduct studies that are similar to those performed in humans.[15] Although highly standardized procedures such as the filamentous MCAO have been developed in rodent stroke research, lesion volume and topology differ between individual mice, for instance, because of variations in the vasculature and degree of collaterals, such as the patency of the posterior communicating artery.[16] In addition, lesion volumes are different between mouse strains.[17] Due to the resulting variance of lesion volumes and topology as well as small sample sizes in rodent stroke research, a systematic analysis of predictors of post-stroke outcome using early non-invasive MRI is challenging. Furthermore, detecting motor-functional deficits in rodents following stroke is difficult as they show impressive recovery and have subtle, if any, long-term motor deficits in common behavioral tests.[18]

In a previous study, we have investigated accuracy of early perfusion/diffusion MRI for final infarct prediction.[19] The main goal of the present study was to identify and compare different predictors of functional outcome following brain ischemia in mice. We therefore aggregated imaging and behavioral data from 13 MCAO studies. Using a newly modified protocol for the staircase test of skilled reaching, we tested forepaw function before and after stroke over a period of 41 days. We defined two phases of post-stroke recovery and tried to predict functional outcome after one week (subacute outcome) or three weeks (long-term outcome) using early MRI. We hypothesized that incorporating lesion topology and behavioral data would improve the predictability of post-stroke outcomes compared to using only the lesion volume. Therefore, we compared the prognostic value of lesion volume and topology for predicting the *subacute deficit* and additionally included the *subacute deficit* for predicting the *residual deficit* using machine learning on a large cohort of 148 mice. We replicated our findings in an independent cohort. Our work opens new avenues towards a better understanding of post-stroke recovery and impairment in mice and paves the way to using prediction of functional outcome for the optimization of preclinical stroke studies.

METHODS

Experimental Design

13 studies from 2016 to 2019 were pooled for behavioral and imaging analyses. Mice were trained in the staircase test for three weeks before and after 45 min of MCAO surgery followed by MRI after 24 hours (Figure 1A, details in Supplemental Materials & Methods). 360 mice were considered for inclusion into this study. Four exclusion criteria were pre-specified (Figure S1): **1)** In the third week of testing, mice performed below 20% of the average in the staircase test (n=30); **2)** no visible infarct in MRI (n=18); **3)** Surgical death or humane euthanasia before study end (n=121) and **4)** incomplete data sets for behavioral data (n=25). Following these criteria, 166 mice were included (Table S1). To validate our prediction models, an additional cohort was built, termed *replication cohort*, using two studies from 2015 and 2019 with n=49 mice finally included (Figure S2). Basic characteristics of the animals are provided in Table S2 and Figure S3.

Staircase Test of Skilled Reaching

Behavioral testers were blinded to the intervention and MRI results. Mice were housed, food restricted and familiarized with the staircase test (details in Supplemental Materials & Methods).[20] We chose one experimental variable, the percentage of collected pellets per side. Performance was expressed as a percentage of average pre-stroke performance (*baseline*). Mice were randomized and allocated to either MCAO or sham surgery. Based on the mean performance of the entire cohort after stroke, two phases were defined: a '*subacute deficit*' phase (days 2–6), during which performance appeared dynamic and increasing, and a '*residual deficit*' phase (days 12–21), throughout which performance appeared stable. Average performance was calculated for each mouse for the respective period. A gap of five days was chosen between the *subacute* and *residual deficit* to ensure the dynamic phase had ended.

Classification of Severity Grades for Subgroup Analysis

Alluding to the modified Rankin scale, a score regularly used in stroke patients, five different functional subgroups were defined.[21] Subgroups were based on the severity of the motor-functional deficit of the paretic paw: mild (performance >80%), moderate-mild (60–79%), moderate (40–59%), moderate-severe (20–39%) and severe deficit (0–19%). Both *subacute* and *residual deficit* were used to define subgroups, depending on the analysis context (Figure 1B–D).

Selection of Predictors

Two factors were analyzed for their predictive value for the degree of the *subacute deficit* after MCAO: **1)** stroke *lesion volume* measured within the coordinates of the Allen Mouse Brain Atlas (AMBA), which makes it identical to the volume of remaining brain tissue,[22] and **2)** MRI lesion percentages measured in the 736 AMBA regions. Throughout the manuscript, this predictor is referred to as *segmented MRI*. The dimensionality of the *segmented MRI* (736 initial regions) was reduced by discarding all regions that did not show a lesion in at least one animal. This left the *segmented MRI* with 536 regions In addition to those two imaging-based parameters, for the prediction of the *residual deficit*, the degree of *subacute deficit* was included as a third factor.

Development of Prediction Models with Machine Learning

For development of machine learning based prediction models, we divided the data set into a training (2/3 of the animals) and a test (1/3 of the animals) group (details in Supplemental Materials & Methods). For each predictor, we calculated the median absolute error (MedAE) and report the average as *prediction error* (*PE*). Only *PEs* from the test cohort were used for accuracy analysis. *PE* measures the difference between predicted and actual performance. Small errors indicate high accuracy. First and third quartiles and interquartile ranges (IQR) of absolute error were calculated to estimate *PE* variation within a cohort. To determine which anatomical regions affect MRI-based *residual deficit* prediction accuracy, the *out-of-bag* predictor importance for each anatomical region in the *segmented MRI* was calculated (details in Supplemental Materials & Methods).[23]

Statistics, Data Availability & Ethics Approval

Data are reported according to the ARRIVE guidelines. Methods for statistics, data display and availability including ethics approval are described in the Supplemental Materials & Methods.

RESULTS

MRI Characterization and Lesion Topology

Total cohort and functional subgroup incidence maps were generated (Figure 2A). Average *lesion volume* was 25.00 mm^3 (95% CI: 21.46–28.54). While heterogeneity of stroke volumes was increased in the 45 minutes compared to a 30 minutes occlusion (Figure S4), it was smaller than in a previously published study performing 60 minutes of MCA occlusion.[15] Classified by degree of residual deficit, mean lesion volume was 14.03 mm^3 (9.82–18.25) in mild, 17.17 mm^3 (12.52–21.82) in moderate to mild, 26.29 mm^3 (16.86–35.73) in moderate, 33.62 mm^3 (24.04–43.20) in moderate to severe, and 47.23 mm^3 (36.79–57.68) in severe residual deficit mice (Figure 2B). One-Way ANOVA showed a significant difference in mean lesion volumes between functional subgroups ($F(4,143)=14.14$, $p<0.0001$; full report in Table S3). T2-weighted MR-images were co-registered on the AMBA to evaluate lesion topology (*segmented MRI*). Quantitative evaluation of the hyperintense lesion was performed on 536 regions and lesion volume in each region was expressed in percent (Table S4).

A Modified Staircase Test Can Assess Early and Long-Term Functional Outcome in Mice after Stroke

Performance peaked after approximately 7 days (Figure 3A–B). The average percentage of retrieved pellets during *baseline* testing was 49.26% (47.22–51.31) on the left (later non-paretic) side and 46.29% (44.16–48.42) on the right (later paretic) side in the MCAO group and 45.81% (37.38–54.23) and 47.11% (38.95–55.26) on the left and right sides in the sham group (Figure S5). Mixed effects analysis showed no side-group interaction ($F(1,164)=1.464$, $p=0.2281$). After MCAO, mean subacute performance was 61.60% (56.12–67.08) on the non-paretic side and 37.59% (31.92–43.26) on the paretic side (Figure 3C). In sham animals, subacute performance was 78.40% (63.46–93.34) on the left and 72.23 (57.30–87.15) on the right side. Mixed-effects analysis found a significant interaction between side and group ($F(1,164)=4.189$, $p=0.0423$). After correcting for multiple comparisons using Šidák's test, MCAO animals had significantly lower paretic paw performance than sham (LSMD=34.63 percentage points [pp], $t=4.092$, $DF=328$, $p=0.0001$). After correcting for multiple comparisons using Šidák's test, performance of the paretic paw was significantly lower in MCAO animals when compared to sham (LSMD=34.63pp, $t=4.092$, $DF=328$, $p=0.0001$). Non-paretic sides did not differ between groups (LSMD=16.80pp, $t=1.985$, $DF=328$, $p=0.0937$). Paired t-test was performed to test for a difference in between paretic and non-paretic paws of the MCAO mice to show side-specificity of the motor-functional deficit. Performance in the paretic paw was significantly lower than in the non-paretic paw (MD: 24.01 %, 18.34–29.68, $t=8.365$, $df=147$, $p<0.0001$) (Figure S6A).

In the period of residual deficit, non-paretic performance was 95.35 % (89.54–101.17) and paretic performance was 60.88 % (54.94–66.81; Figure 3D). During the phase of residual deficit, sham animals performed 92.39% (81.80–102.98) on the non-paretic side and 91.56% (78.45–104.66) on the paretic side. Mixed-effects analysis showed a significant interaction between side and group ($F(1,164)=9.384$, $p=0.0026$). Using Šidák's test, paretic paw performance was significantly lower in MCAO animals compared to sham (LSMD=30.68pp, $t=3.501$, $DF=328$, $p=0.0011$). Non-paretic sides showed no statistically significant difference between groups (LSMD=2.96pp, $t=0.3377$, $DF=328$, $p=0.9302$). Finally, we analyzed the effects of the general well-being of the animals on functional outcome. We used the Modified

DeSimoni Neuroscore for the assessment of general deficits and the weight loss on day 2 as proxies for general well-being and found poor correlation to the degree of either subacute or residual deficit (Figure S7 and S8).[24] In addition, *PEs* of weight loss or neuroscore were significantly higher compared to imaging- or performance-based prediction (Figure S9 and S10).

Prediction of Subacute Deficit from Acute MR Imaging

Using random forest and early non-invasive MRI data, *subacute deficit* prediction models were created. *PE* was 13.30pp (13.00–13.59, Q_1: 5.98, Q_3: 31.48, IQR: 25.51) for the *lesion volume* and 16.23pp (16.03–16.42, Q_1: 8.04, Q_3: 29.18, IQR: 21.14) for the *segmented MRI* (Figure 4A–B). Unpaired, two-tailed t-test revealed, that *PE* was significantly smaller when using the *lesion volume* as a predictor compared to the *segmented MRI* (MD=2.93pp, 2.58–3.28, t=16.60, df=98, p<0.0001).

Prediction of Residual Deficit from Subacute Deficit and MRI

PE was 18.90pp (18.70–19.09, Q_1: 8.04, Q_3: 29.09, IQR: 21.05) for the *subacute deficit*, 22.68pp (22.46–22.90, Q_1: 14.07, Q_3: 41.84, IQR: 27.77) for the *segmented MRI* and 25.95pp (25.71–26.29, Q_1: 12.21, Q_3: 41.25, IQR: 29.04) for the *lesion volume* (Figure 4C–D). One-way ANOVA showed predictors differed significantly (F(2,147)=1032, p<0.0001). After multiple comparisons were corrected using Tukey's test, the *PE* of the *subacute deficit* was significantly smaller than *PE* of the *segmented MRI* (MD=3.79pp, 4.15–3.42, p<0.0001) and the *lesion volume* (MD=7.06pp, 7.43–6.69, p<0.0001). *Segmented MRI* outperformed the prediction accuracy of the *lesion volume* (MD=3.28pp, 3.65–2.91, p<0.0001).

Identification of Anatomical Regions Associated with Long-Term Outcome

To better understand which anatomical regions within the lesion best predict long-term outcome, the contribution of each region measured by MRI was analyzed by quantifying its importance within the random forest algorithm. We found 81 regions with above-zero out-of-bag predictor importance, meaning they contribute more than random data (Figure 5A–B, Table S5). Among those regions were the caudoputamen, the primary somatosensory area, the external capsule and the corticospinal tract. In the next step, random forest models were trained using increasing number of MRI regions, starting with the most important region. The *PE* of those models gradually decreased with increasing number of MRI regions (Figure 5C). *PE* quickly reached a plateau, and adding more regions only slightly improved accuracy. A relevant local minimum of *PE* was observed with the first 14 anatomical regions (*PE*=22.91pp).

Validation in an Independent Cohort

As a final step, we tested our prediction models on the independent *replication cohort*. Imaging and behavioral analyses can be found in the Supplemental Materials & Methods. For the prediction of the *subacute deficit*, mean *PE* when using the *lesion volume* was 23.19pp (22.85–23.53) and 20.00pp (19.74–20.27) for the *segmented MRI* (Figure 6A–B). In contrast to our first cohort, now the *segmented MRI* provided significantly smaller *PEs* compared to the *lesion volume* (MD=3.19pp, 3.61–2.76, t=14.89, df=98, p<0.0001). The *PE* for the *residual deficit* using the *subacute deficit* was 10.76pp (10.31–11.22), 22.96pp (22.15–23.77) for the *segmented MRI* and 23.94pp (23.41–24.46) for the *lesion volume* (Figure 6C–D). One-

way ANOVA revealed, that there was a statistically significant difference between predictors ($F(2,147)=18.98$, $p<0.0001$). After correction for multiple comparisons using Tukey's test, the *PE* of the *subacute deficit* was significantly smaller than the *PE* of the *segmented MRI* (MD=12.20pp, 13.23–11.17, $p<0.0001$) and the *lesion volume* (MD=13.18pp, 14.20–12.15, $p<0.0001$). The difference between s*egmented MRI* and the *lesion volume* was less clear than in our first cohort, but trended towards significance, with the *segmented MRI* providing slightly smaller PEs compared to the *lesion volume* (MD=0.98pp, 2.01–0.05, $p=0.0668$). Lastly, we tested the prediction accuracy when including an increasing number of anatomical regions. Only regions we identified to have an impact on the prediction of the *residual deficit* in our *testing cohort* were used. Surprisingly, in our *replication cohort*, using the lesion percentage in the caudoputamen provided smaller *PE*s compared to using the entire *segmented MRI* or the *lesion volume*. When adding an increasing number of regions, we first observed a sharp spike in *PEs*, followed by a slow decrease. Excitingly, after including fourteen regions, we again observed a local minimum of *PE* (Figure 6E), replicating our finding from the *testing cohort*.

DISCUSSION

In this study, we systematically compared different predictors of subacute and long-term functional outcomes after stroke in a large cohort of mice. We used MRI derived parameters such as the *lesion volume* and *lesion topology*, as well as early functional testing to predict post-stroke performance in a pellet reaching task. Studies with large sample sizes have transformed preclinical MRI research and corresponding open data repositories have proven extremely valuable, e.g. in the context of robust functional networks measured by resting state fMRI.[25] Predictors of post-stroke outcome, such as imaging-derived parameters or functional data, have previously been extensively studied in humans.[2–6,8] While one preclinical study on predictors of stroke outcome in a porcine MCAO model exists, evidence on prediction of stroke outcome in mice is scarce, despite rodents being used most commonly as model animals in stroke studies.[12,26]

Using a modified protocol for the staircase test of skilled reaching in mice, we were able to monitor motor function daily and to detect both subacute and long-term functional deficits.[20,27] A side-specific disability of the forepaw was detected over three weeks post-surgery. This indicates that performance was governed by the stroke and not by general sickness behavior. We observed distinct trajectories of functional recovery. The differentiation between recoverees and non-recoverees is comparable to what can be observed in humans.[14] The resemblance to the human recovery process supports the predictive value of stroke modeling in mice and the case for implementing sophisticated behavioral tests that involve a longer phase of training prior to stroke for assessing long-term impairments.[28] For future applications, one has to consider the possible impact of food restriction included as part of the staircase training and testing on mortality and lesion volume.[29]

The *lesion volume* was the best predictor for the *subacute deficit* after stroke in mice as measured by *PE* in our *testing cohort*. In the *replication cohort* it was outperformed by the *segmented MRI*, which included information about the lesion topology. While *PEs* remained low, this is clearly a difference between the two cohorts. However, we also observed differences in stroke size and topology between the two cohorts. In our *replication cohort* lesion volumes were bigger and cortical regions accounted for higher proportions of the *lesion volume* compared to the *testing cohort*. While it has previously been stated that lesion volume functions as a good predictor for acute stroke outcome, our data suggests that in mice with greater cortical lesions taking into account topological information provides a more accurate prediction of early functional outcome.[6] For the long-term motor-functional outcome, in the *testing cohort* the *segmented MRI* provided significantly better prediction accuracies than the *lesion volume*. This indicates that for functional recovery, lesion topology is more relevant than lesion volume, which again is in line with clinical data.[11,12] It should be noted, that in the 45 min MCAO model, lesion volume and lesion topology are not fully independent predictors. Larger ischemic lesions typically include cortical regions while smaller lesions are restricted to subcortical structures. This phenomenon can be observed in the results of our *replication cohort*, where the difference between the *segmented MRI* and the *lesion volume* was only marginal but trending towards significance.

Following up on the influence of lesion topology, we identified anatomical regions that drive imaging-based prediction of the *residual deficit*. We found that considering only a subset of anatomical regions can explain functional long-term outcome. Prediction accuracy displayed a local maximum after 14 regions had been added to the prediction model. Among those regions were both, cortical and subcortical regions typically affected by a MCAO stroke such

as the caudoputamen and the primary somatosensory area. Evidently, the caudoputamen is damaged and part of the lesion core in the transient occlusion model but also serves as the structure that best predicts the performance. Further, white matter regions such as the corpus callosum and the fiber tracts showed to be of particular importance for the prediction of long-term outcome. This is in line with previous studies in humans suggesting that lesions of the corticospinal tract seem to be more important to both early and long-term functional outcome than lesion volume.[30,31] These findings could be replicated in our independent *replication cohort*, where a local minimum of *PE* could be observed after including the same 14 regions. Again, lesions in the caudoputamen had a major impact on prediction accuracies, outperforming both cortical grey and white matter structures.

Imaging-based predictors offered good estimates of post-stroke long-term outcome but the best predictor of the *residual deficit* was the degree of the *subacute deficit*, again confirming studies on predictors of post-stroke outcome in humans.[32] Lastly, we found that independent of the chosen model, the prediction accuracy for both the *subacute* and *residual deficits* depended on the degree of the deficit. In line with clinical data, we found that severe *residual deficits* are more difficult to predict than moderate-to-mild deficits.[13,14]

Study Limitations

We have restricted our prediction tools to 45 min of MCAO using endoluminal filament occlusion. As the data was pooled from 13+2 studies performed between 2015 and 2019, the dataset contained various co-variates. However, variance analysis revealed that sex, genotype, surgeon, or age did not significantly explain differences in performance after stroke. We cannot exclude confounding effects of housing, lab chow diet, anesthesia, or further co-variates. Furthermore, we did not replicate our results with other behavioral readouts than the staircase test of skilled pellet reaching. Additionally, using MR imaging from a later time point, e.g. 21 days after MCAO, would have likely resulted in even better prediction accuracies. However, prediction of functional outcome that is based on late MR-imaging cannot be used for guiding intervention decisions in future preclinical studies as the window of opportunity in mice has proven to be narrow.[33] Another limitation of our approach is the manual delineation of lesion masks. Although manual lesion segmentation is still the gold standard today, well-documented, multi-centric and openly available stroke T1-weighted MRI datasets pave the way towards fully automated algorithms for MRI stroke lesion segmentation in humans.[34–36] Results from T2-weighted MRI in rodent stroke models are also promising with excellent similarity between manual segmentation and fully automated algorithms exceeding agreement between multiple raters.[37,38] These studies have facilitated significant progress in the pursue to fully automate lesion segmentation.[38,39] Nulling of the fluid signal via e.g. FLAIR MRI could have improved the discrimination between CSF and ischemic lesion. However, 24 hours after MCAO, CSF has still higher signal on RARE than the lesion and the walls of the lateral ventricles can be reliably identified.[22] For later time points, an adjusted imaging protocol might have to be applied. Animals' unspecific sickness behavior during the *subacute deficit* phase may have contributed to the degree of performance loss. The differences between sham and MCAO, as well as paretic and non-paretic paw, were, however, significant, indicating that our behavioral paradigm detects stroke-specific motor-functional deficits. There were no video recordings of the testing sessions to account for differences in kinematics but the entire behavioral data set has been uploaded in the repository linked to this study. Analysis of lesion volumes stratified by functional subgroups might be partly

weakened by experimental bias, since lesion volume and performance are strongly correlating. Finally, we corrected only for multiple tests in a single test set and not for multiple comparisons between different tests or hypotheses.

CONCLUSIONS

In conclusion, we have here for the first time identified and characterized predictors of post-stroke outcome in mice. The concordance with clinical data strengthens the usability of mice as model animals in preclinical studies. Prediction of outcome in intervention studies using non-invasive MRI measurements or motor-functional data should be utilized to compare expected and achieved motor-function outcomes, select mice with particularly poor predicted outcomes, and guide treatment or intervention options. This will help to improve the reproducibility of preclinical rodent stroke research and close the translational gap.

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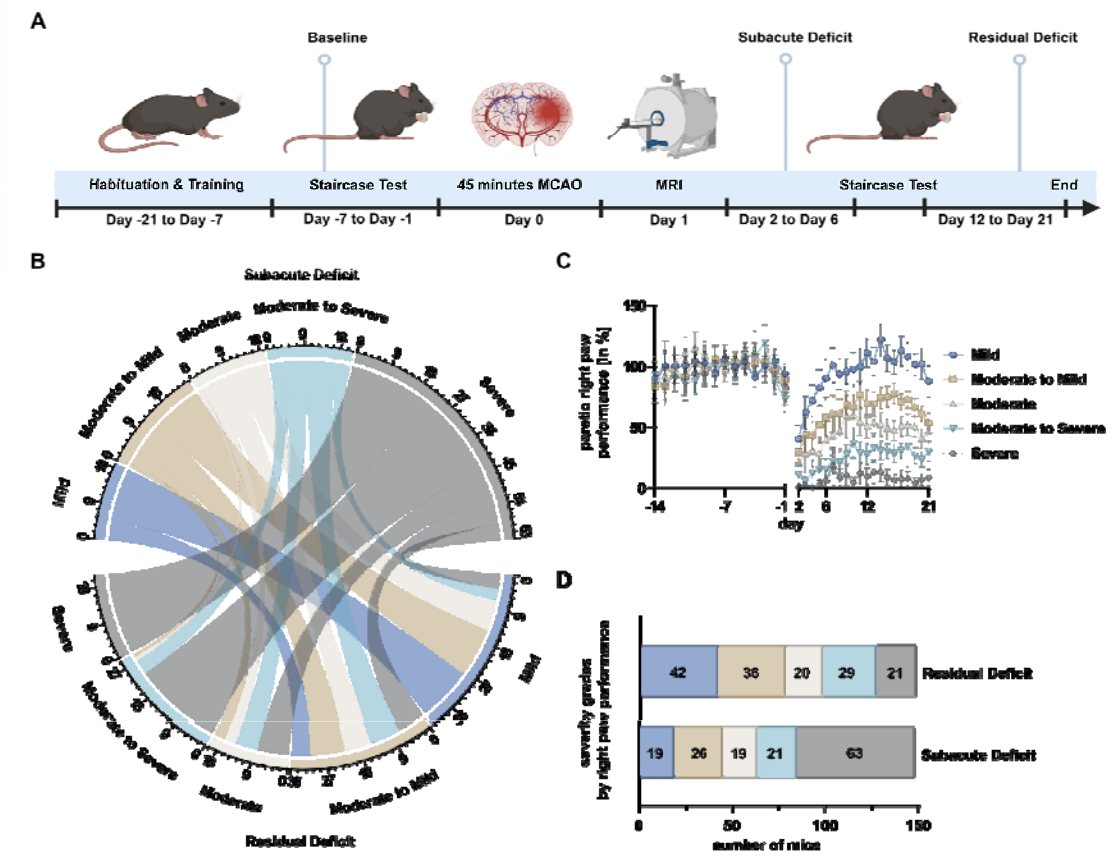

Figure 1. Experimental design and assessment of functional subgroups

A, Mice were trained in pellet reaching for 21 days. Average percentage of retrieved pellets during the last week prior to MCAO was used to normalize performance (baseline). Mice received either 45 min of MCAO or sham surgery. MRI was performed on day 1 and mice were then tested for another 21 days. **B**, Chord diagram displaying the different trajectories of recovery. Groups on top display mice stratified by degree of *subacute deficit*, while groups on bottom display mice stratified by *residual deficit*. Numbers indicate group sizes. Interconnections visualize how many mice with a given *subacute deficit* recovered to have a given *residual deficit*, i.e. roughly a third of the mice with a severe *subacute deficit* displayed no recovery and showed a severe *residual deficit*. **C**, Performance of the paretic right paw over time for each severity grade. Error bars indicate 95% CI. Severity grades here were based on the degree of the *residual deficit*. **D**, Overview over sizes of functional subgroups. Groups are either based on the degree of *subacute* or *residual deficit*. While the majority of mice displayed a severe *subacute deficit*, due to their impressive capability for spontaneous recovery, the majority of mice displayed only mild *residual deficits*.

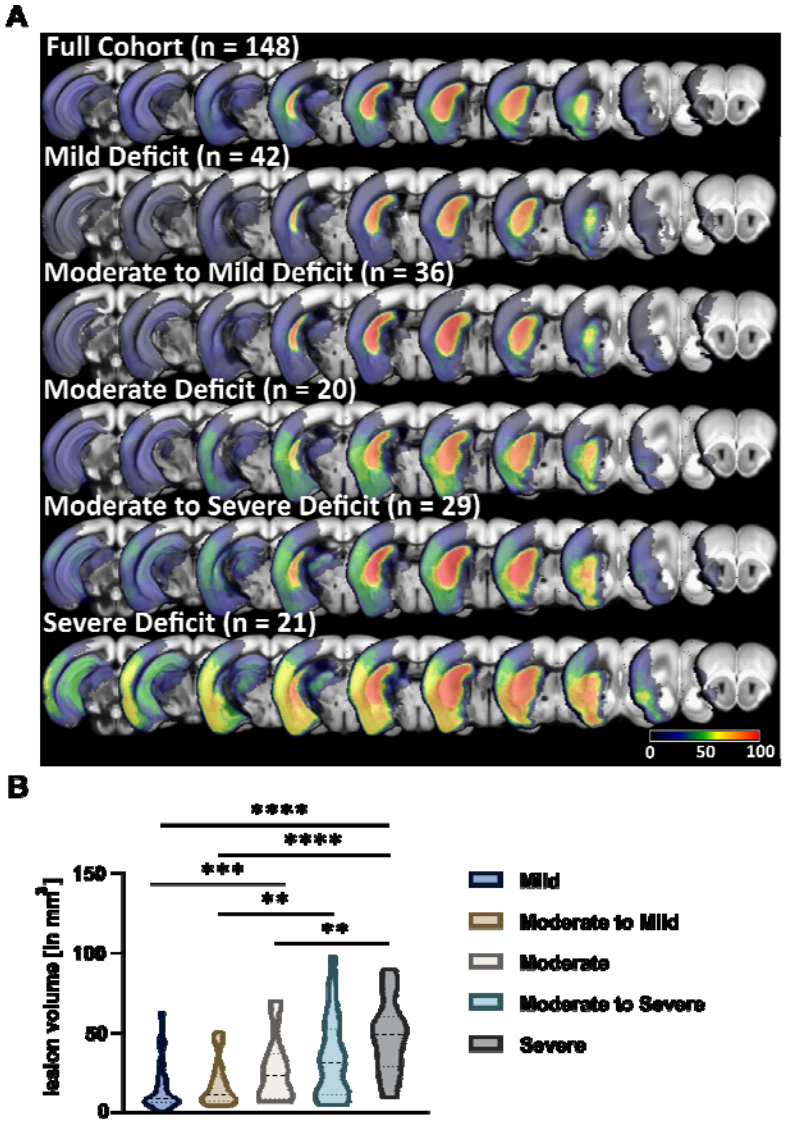

Figure 2. MRI characterization of lesion volume and location.
A, Coronal incidence maps of stroke lesions in the total cohort and the five different functional subgroups. Colours indicate percentage of mice with a lesion in a given region. Severity grades are based on degree of *residual deficit*. **B**, *lesion volume* by severity grade (based on degree of *residual deficit*). Great variability within functional subgroups could be observed and there was no significant difference when comparing *lesion volume* of consecutive severity grades, i.e. of mice with mild or moderate to mild *residual deficits.*

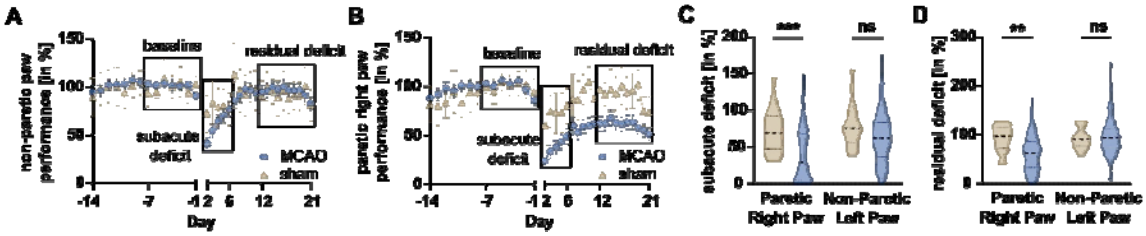

Figure 3. Assessing the sensorimotor deficit after stroke.

A, Non-paretic left and **B**, paretic right paw performance over time, bars depict 95% confidence interval. Performance on days -7 to -1 were averaged and taken as a baseline (first black rectangle). Data from days 2 to 6 were summarized and referred to as *subacute deficit* (second rectangle) while data from days 12 to 21 are referred to as *residual deficit* (third rectangle). **C**, *Subacute* and **D**, *residual deficit* of the non-paretic and paretic paw in sham (brown plot) and MCAO (blue plot) animals. Dashed lines indicate medians, dotted line indicate quartiles. Mixed-effects model followed by Šidák's post-hoc tests for multiple comparison were used to test effect of group and side.

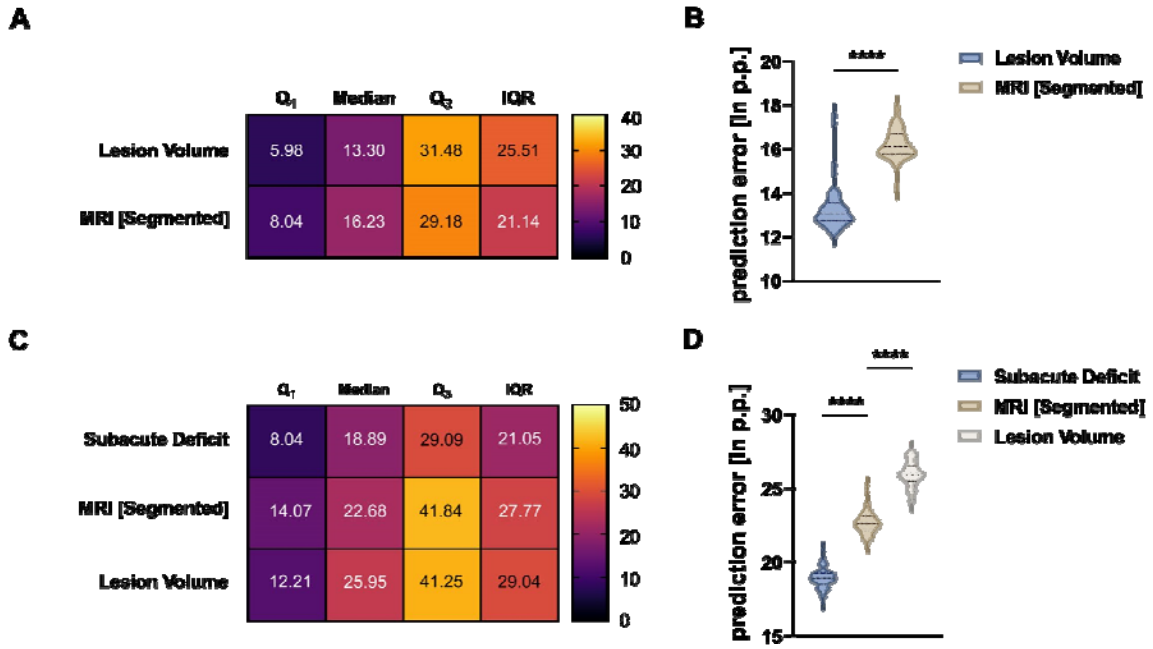

Figure 4. Comparison of predictors of the subacute and residual deficit.
A through **B**, Comparison of the prediction accuracy of the two predictors of the *subacute deficit*. Averaged Q_1, median, Q_3 and IQR were calculated using the 50 independent models for each predictor. Dashed lines indicate median, dotted lines indicate quartiles. Unpaired t-test was used to compare *PEs*. Small *PE* indicates small deviation between predicted and achieved performance. **C** through **D**, Comparison of prediction accuracy of the two imaging based parameters and the *subacute deficit* for the prediction of the *residual deficit*. Again, one-way ANOVA followed by Tukey's test for multiple comparison was used.

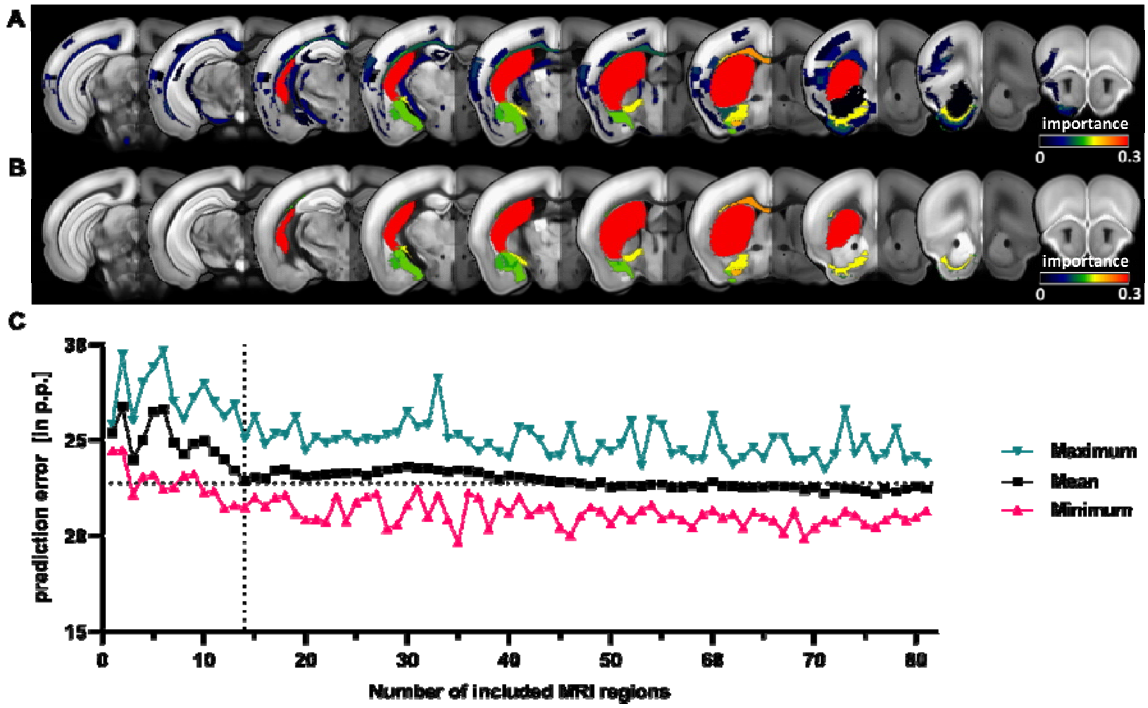

Figure 5. Identification of anatomical regions that drive imaging-based prediction of functional outcome.

A, Coronal slices depicting the importance of the 81 regions calculated from our random forest models (RF). Only regions with an importance level >0 are shown **B**, Visualization of the importance level of the 14 anatomical regions that were included in the prediction model that resulted in a local minimum of *PE*. **C**, Prediction of *residual deficit* was performed using an increasing number of regions in the *segmented MRI*. *PE* as well as maximum and minimum *PE* are depicted. Dashed lines at x=14 and y=21.5 denote the first local minimum of *PE* when including only 14 regions.

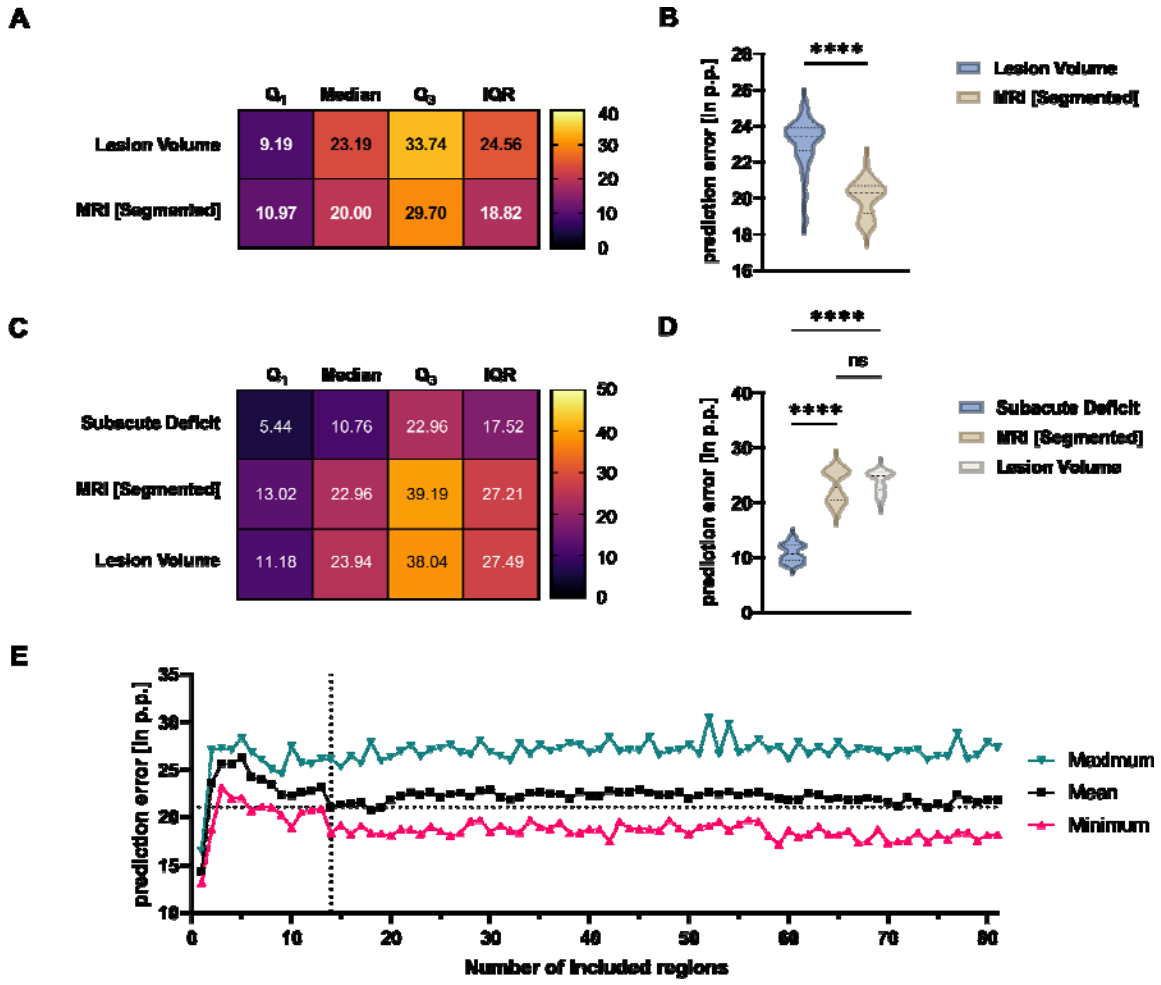

Figure 6. Validation of prediction models in an independent cohort.
A, through **B**, Comparison of the prediction accuracy of the two predictors of the *subacute deficit*. Dashed lines indicate median, dotted lines indicate quartiles. Unpaired t-test was used to compare *PEs*. While *PEs* were comparable to the ones seen in our testing cohort, subacute deficit was best predicted when using the segmented MRI **C** through **D**, Comparison of prediction accuracy of the two imaging based parameters and the *subacute deficit* for the prediction of the *residual deficit*. Again, the *subacute deficit* was the best predictor of the *residual deficit*, followed by the *segmented MRI* and the *lesion volume*. **E**, Analogous to the testing cohort, prediction of *residual deficit* was performed using an increasing number of regions in the *segmented MRI*. Dashed lines at x=14 and y=21.5 denote the local minimum of *PE* that had previously been observed in the testing cohort.