** EBRAINS Data Descriptor**

*The HBP Data Descriptor follows closely the data descriptor template of the journal Scientific Data (ISSN 2052-4463, https://www.nature.com/sdata/). Note that sections marked with an asterisk are obligatory.*

**TITLE\***

*110 characters maximum, including spaces, no colons and parentheses*

*The title should be descriptive for the presented data. The use of acronyms and abbreviations should be avoided where possible.*

Partial restoration of Dp427 in the brain of adult *mdx52* mice after intracerebroventricular injection of ASO targeting *Dmd* exon 51

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**ABSTRACT\***

*170 words maximum, no references*

*The abstract should concisely describe the study, the assay(s) performed, the resulting data, and their reuse potential, but should not make any claims regarding new scientific findings.*

The exon-52-deleted *mdx52* mouse has emerged as a critical model of Duchenne muscular dystrophy (DMD), as it holds a deletion in a hotspot region of the dystrophin gene frequently mutated in patients and is eligible for preclinical studies based on exon-skipping treatment strategies. Deletion of exon 52 impedes expression of several dystrophins expressed in the central nervous system from distinct promoters (Dp427 and Dp140 in the brain, Dp260 in the retina). In this study, we examined the possibility to restore Dp427 expression in the brain of *mdx52* mice using exon 51 skipping. For that purpose, we performed intracerebroventricular (ICV) administration of tricycloDNA (tcDNA) antisense oligonucleotides (ASO) targeting exon 51 in 7-wk-old male *mdx52* mice. In a first study (**named AS-07**), mice were analyzed at different time points after the injection (3, 7 and 11 wks post-injection) to assess the level of exon 51 skipping in different brain regions and the associated levels of Dp427 restoration. We showed that ICV injection of 400 µg of tcDNA-ASO targeting exon 51 induces between 16 and 34% of exon 51 skipping in the different brain regions (cerebellum, hippocampus and cortex) and that these levels are stable between 3 and 11 wks post injection. We detected Dp427 restoration from 7 weeks after the ICV injection, with levels ranging from 2 to 10% (of WT levels) in the different brain regions. Expression of Dp427 was stable between 7 and 11 wks post injection. The fear response was also analyzed in these mice (despite the small n number that had been calculated for molecular analysis) and revealed a tendency to improvement at 7 wks post administration (compared to control *mdx52* mice) although not statistically significant. In a second study with higher n number (**named AS-08**), mice were treated similarly (ICV injection of 400 µg of tcDNA-ASO targeting exon 51 at 7 weeks of age) and analysed for behavioral outcomes between 7 and 9 weeks post injection. Anxiety and unconditioned fear response were significantly improved in treated *mdx52* mice during this period. Moreover, acquisition of fear conditioning was fully rescued, while fear memory tested 24h later was only partially improved. Additional restoration of Dp427 in skeletal and cardiac muscles by systemic treatment (**study named AS-09**) did not further improve the unconditioned fear response, confirming the central origin of this phenotype. These findings indicate that some emotional and cognitive deficits associated with dystrophin deficiency may be reversible or at least improved by partial postnatal dystrophin rescue.

**BACKGROUND & SUMMARY**

*700 words maximum, optional section*

*This section should provide a more detailed overview of the study, the assay(s) performed, and the resulting data as well as referencing previous work and related literature to put the study into context. This section should also briefly outline the broader goals that motivated collection of the data, as well as their potential reuse value.*

Duchenne Muscular Dystrophy (DMD) is a neuromuscular disease that affects 1:5000 male births and is associated with non-progressive cognitive, behavioral and neuropsychiatric comorbidities.1–3 DMD is caused by mutations in the dystrophin (*DMD*) gene that encodes multiple dystrophin proteins (Dp). Dystrophins are membrane-bound proteins involved in receptor and ion channel clustering in a cell and tissue-specific manner. The dystrophins differ by their molecular weight, expression, and function. The full-length dystrophins, Dp427M/C/P, are expressed in muscles (Dp427M) as well as in central GABAergic synapses in brain (Dp427C) and cerebellum where they contribute to the synaptic clustering of GABAA receptors.4,5 The smaller C-terminal brain dystrophins are expressed from independent internal promoters: Dp260 is selectively expressed in retina, Dp140 shows enriched expression in the fetal human brain but its cellular localization in adult brain is still unclear5,6, and Dp71 is expressed in excitatory synapses as well as in astrocyte endfeet forming the blood brain barrier (BBB), where it plays a role in aquaporin 4 (AQP4) regulation.7 Mutations in the *DMD* gene lead to muscular dystrophy due to the loss of the muscle dystrophin, while the nature and severity of brain alterations in DMD patients depend on the position of the mutation and on the type and number of dystrophins affected by the mutation.3 While proximal mutations inducing the loss of the full-length Dp427 are generally associated to very modest effect on cognitive function, the more distal ones are associated with more severe deficits due to the cumulative loss of several brain dystrophins.8,9 Functional studies of different DMD mouse models provided an essential contribution to our understanding of the affected brain mechanisms depending on the position of the mutation and loss of different dystrophins.10–12 We previously demonstrated that the exon52-deleted *mdx52* mouse model,13 lacking Dp427, Dp260 and Dp140 shows stronger emotional alterations as compared to the original Dp427 deficient-*mdx* mouse model.10 Indeed, the mutation is located in a “hot spot” region frequently found to be mutated in DMD patients (65%).1,3,9 The development of therapeutic approaches in this mouse model is thus of great interest, as it directly translates to patients’ condition. One of the most promising therapeutic strategies for DMD aims to restore the open reading frame in order to express an internally deleted but still functional protein. This so-called exon-skipping strategy is based on the use of antisense oligonucleotides (ASOs) that interfere with splicing signals or regulatory elements in the exon or intron, thus leading to the skipping of the targeted exon at the precursor (pre-)mRNA level.14–16 Previous studies in *mdx52* mice demonstrated the therapeutic potential of the exon-skipping approach to restore expression of Dp427 in muscles using naked ASO17 or vectorized sequences in AAV-U7snRNA vector.18 The feasibility of antisense-based therapies has been demonstrated in clinical trials and several ASO drugs have now been conditionally approved by the FDA.19 However, none of the currently approved ASO drugs are capable of addressing DMD brain comorbidities, mostly because of their inability to cross the BBB. Yet novel ASO chemistries or conjugates are currently being developed and may offer promising tools to treat both the dystrophic phenotype and the central deficits associated with the lack of brain dystrophin. Among these, we have previously demonstrated that tricyclo-DNA (tcDNA)-based ASOs display unprecedented uptake in many tissues including cardiac muscle and central nervous system (CNS) after intravenous administration in mouse models of DMD20–22 and SMA.23 More recently, we have shown that local administration of tcDNA-based ASO in the brain of *mdx* mice lacking only Dp427 alleviates some cognitive deficits associated with DMD.24

In the present study, we aimed to investigate the impact of postnatal restoration of brain Dp427 in the more severe *mdx52* model, which is representative of a larger subpopulation of DMD patients. For this purpose, we used a tcDNA-ASO conjugated to palmitic acid22 and targeting dystrophin exon 51, in order to restore Dp427 exclusively. Given that exon 51 contains the start codon for Dp140, skipping of exon 51 indeed cannot restore Dp140 expression. We first determined the optimal therapeutic window following intracerebroventricular (ICV) microinjection of tcDNA-Ex51 and then assessed its potential in rescuing behavior, using tests in which *mdx52* mice typically show deficits.10 Anxiety, unconditioned fear and conditioned fear learning and memory were successively assessed in treated *mdx52* mice. The efficacy of the treatment was also analyzed at the molecular level, and we further evaluated the contribution of partial Dp427 restoration by systemic injection in muscles and heart, to rule out the possibility of a peripheral contribution to the behavioral improvements observed.

**METHODS\***

*This section should be used to describe any steps or procedures that produced the data, including full descriptions of the experimental design, data acquisition assays, and any computational processing (e.g., normalization, image feature extraction). Each method should be described under correspondingly named subheading. It is acceptable to cite previous descriptions of the methods under use, but the descriptions should be complete enough for others to understand and reproduce the methods and processing steps without reading the associated publications. There is no word limit to the length of this section.*

The methods used to produce the data have been described previously in 10,18:

Saoudi A, Zarrouki F, Sebrie C, Izabelle C, **Goyenvalle A**, Vaillend C. Emotional behaviour and brain anatomy of the mdx52 mouse model of Duchenne muscular dystrophy. Dis Model Mech. 2021 Sep 1;14(9):dmm049028. doi: 10.1242/dmm.049028

Aupy P, Zarrouki F, Sandro Q, Gastaldi C, Buclez PO, Mamchaoui K, Garcia L, Vaillend C, Goyenvalle A. Long-Term Efficacy of AAV9-U7snRNA-Mediated Exon 51 Skipping in mdx52 Mice. Mol Ther Methods Clin Dev. 2020 May 4;17:1037-1047. doi: 10.1016/j.omtm.2020.04.025. PMID: 32462052; PMCID: PMC7240049.

**Experimental design study AS-07**:

Adult (6-8-wks old) male *mdx52* mice received an intracerebroventricular (ICV) injection of 400 ug of tcDNA-ASO targeting exon 51 (group AS-07 treated, n=15, mouse ID: T01-T15) or saline control (group AS-07 PBS, n=12, mouse ID: PBS01 – PBS12). Age and gender matched littermate wild-type controls were used as controls (group AS-07 WT, n=10, mouse ID: WT01 – WT10).

Mice were analyzed at three different time points:

* 3 weeks after the injection
	+ group AS-07 treated, n=5, mouse ID: T11-T15
	+ group AS-07 PBS, n=4, mouse ID: PBS09 – PBS12
	+ group AS-07 WT, n=3, mouse ID: WT08 – WT10
* 7 weeks after the injection
	+ group AS-07 treated, n=5, mouse ID: T06-T10
	+ group AS-07 PBS, n=4, mouse ID: PBS05 – PBS08
	+ group AS-07 WT, n=3, mouse ID: WT04 – WT06
* 11 weeks after the injection
	+ group AS-07 treated, n=5, mouse ID: T01-T05
	+ group AS-07 PBS, n=4, mouse ID: PBS01 – PBS04
	+ group AS-07 WT, n=2, mouse ID: WT01 – WT02

**Experimental design study AS-08**:

For the more extent behavioral study (AS-08), 3 groups of mice were used and underwent surgery in identical conditions. Eight-week-old *mdx52* male mice were treated with the tcDNA-Ex51 (n=13, mouse ID T01-T09) or with the tcDNA-sense as a control (n=10, mouse ID C01-C12), while WT littermate males were treated with saline (n=13, mouse ID WT01-13). Seven weeks after ICV injections, within a therapeutic window of 2 weeks, the mice were tested in a battery of behavioral tests in the following order: elevated plus maze (EPM), light/dark choice (LDC) test and restraint-induced unconditionedfear with 24h interval. The order of the tests was specifically chosen to minimize their influence on each other. A week of gentle handling preceded the auditory-cued fear conditioning, to reduce stress before testing. Behavioral testing was performed blind to the genotype.

All mice were euthanized at the end of the behavioral testing, i.e. 9 weeks after the ICV injection and brain samples were collected for further molecular analysis (% of Exon 51 skipping, WB Dp427).

**Experimental design study AS-09**:

For the treatment combining systemic and central injections (AS-09), a separate group of 5 week-old *mdx52* male mice (n=8, mouse ID T01-T08) underwent intravenous injections of 3 E+14 vg of scAAV9-U7Ex51M. Three weeks later, half of them also received tcDNA-Ex51 by ICV and the other half received PBS. Seven weeks after the ICV injections, these animals, their non-injected WT littermates (n=5 mouse ID WT01-WT05) and their *mdx52* littermates IV injected with PBS (n=5, mouse ID PBS01-PBS05) were submitted to restraint-induced unconditioned fear.

Twenty-four hours later, all mice were euthanized and brain and muscles samples were collected for further molecular analysis (% of Exon 51 skipping, WB Dp427).

**TECHNICAL VALIDATION\***

*This section should present any procedure that is needed to support the technical quality of the data. It should justify the reliability of the presented data. This may include: experiments supporting or validating the data-collection procedure, statistical analyses of experiment errors and variation, phenotypic or genotypic assessments of biological samples, any procedure used to ensure reliable and unbiased data production, acclimatisation procedures to ensure the protection of personal data, etc.. This should not include: follow-up experiments aimed at testing/supporting an interpretation of the data, statistical hypotheses testing, or exploratory computational analyses like clustering and annotation enrichment.*

**USAGE NOTES\***

*This section should contain brief introductions to assist others with reuse of the presented data. This can include suggestions of software packages that are suitable for analyzing the presented data, or tips for further processing steps and for integrating or compare the presented data with other data.*

*Under a subsection called “SPATIAL ANCHORING”, information should be provided that facilitates the spatial anchoring of the presented data into the HBP interactive atlas viewers. If possible, brain image data and coordinates should be provided not only in native space, but in one of the following brain reference spaces: BigBrain template [v1], MNI Colin27 [v1], MNI ICBM 152 [2009c, nonlinear, asymmetric], Infant brain template [v4.0], Allen Mouse CCF [v2 or v3], WHS SD atlas template [v2.0]. Semantic links preferred to brain regions of one of the following atlases: BigBrain parcellation [v1], JuBrain probabilistic cytoarchitectonic atlas [v18], Infant brain atlas [v4.0], Allen Adult Mouse Brain Reference Atlas [v2 or v3], WHS SD atlas [v2.0].*

All behavioral data were analyzed using the software ANY-maze (Stoelting Co).

**SPATIAL ANCHORING:**

**DATA RECORDS\***

*This section should be used to explain the data presented in this descriptor and the repository where they are stored. This should include an overview of data files and formats, and potentially a short content description and file-internal data structure for each file type. Example of a mock-up repository overview:*

In the repository the data are stored in the following structure (incl. info on file content):

**repository-root/**

**data-descriptor.pdf** *[contains a short description of the dataset*

**sub\_info.tsv** *[contains information on the subjects*

**experimental-methods\_info.json** *[contains information on the applied experimental method]*

**sub-XXX/**

**sub-XXX\_slice-XXXX.tif** *[brain slice scan of subject XXX; index XXXX equals physical slice position in µm, anterior to posterior]*

**derived-data/**

**analysis-methods\_info.json** *[contains information on the applied analysis methods]*

**analysis-X/**

**analysis-X\_set-X.tsv** *[contains result data of analysis X]*

**code/**

**analysis-X.py** *[script that produces result data of analysis X]*

Information on used file formats and file-internal data structures:

**Tab-Separated Value format (tsv):** *labels in first row; data of same type in columns*

**JavaScript Object Notation (json):** *nested key-value pairs [cf. templates in xx]*

**Tagged Image Format File (tif):** *100µm thickness; 1x1 pixel dimension; unit in µm*

**Python Script (py):** *Python 3.0 script*

In the repository, the data are stored under an excel format.

The datasheet ‘partial restoration of dystrophin’ contains 10 sheets:

1- Summary of metadata (AS-07, AS-08, AS-09)

2- AS-07 behavioral study

3- AS-07 % of Ex51 skipping

4- AS-07 WB Dp427

5- AS-08 behavioral study

6- AS-08 % of Ex51 skipping

7- AS-08 WB Dp427

8- AS-09 behavioral study

9- AS-09 % of Ex51 skipping

10- AS-09 WB Dp427

In each sheet, the subject and the tissue samples are organized in lines.

**CODE AVAILABILITY\***

*This section should list all software / code (ready-made or custom-made) and their version used in the generation or processing of the presented data. This should include a statement indicating whether and how the software or code can be accessed, including any access restrictions. If relevant, provide also any specific variables or parameters used to run the software / code on the presented data.*

All behavioral data were analyzed using the software ANY-maze (Stoelting Co).

**Acknowledgements**

*This section should contain brief acknowledgements of non-author contributors. Anonymous referees and editors or effusive comments are not accepted. Grant or contribution numbers can be acknowledged.*

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**Author contributions**

*This section should state briefly on a separate line each author’s contribution to producing or maintaining the presented data as well as their role in publishing them.*

Conceptualization, C.V., F.M. and A.G.; Methodology, A.S., C.V. and A.G.; Investigation, A.S., S.B., O.L-E., O.V., M.D.C., T.T., and E.S.; Writing – Original Draft, A.S., C.V. and A.G; Writing – Review & Editing, F.M., C.V. and A.G; Funding Acquisition, F.M., C.V., L.G. and A.G; Supervision, C.V. and A.G.

**REFERENCES\***

*This section should list all bibliographic information for all literature cited in the above sections using the standard Nature referencing style.*

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