Behavioural impairments in a mouse model of Kabuki syndrome associated with dopaminergic and neuroinflammatory modulations

Thalles F. Biondi[1], Silvia M.G. Massironi[2], Eduardo F. Bondan[1] and
Thiago B. Kirsten[1]

[1]Psychoneuroimmunology Laboratory, Program in Environmental and Experimental Pathology, Paulista University, São Paulo, Brazil and [2]Department of Pathology, School of Veterinary Medicine and Animal Science, University of São Paulo, Brazil

Abstract

Objective: Kabuki syndrome is a rare multisystem congenital disorder characterised by specific facial malformations and several other symptoms, including motor impairments, increased susceptibility to infections, immune mediators' deficits, anxiety, and stereotyped behaviours. Considering the reports of motor impairments in Kabuki syndrome patients, the first hypothesis of the present study was that this motor dysfunction was a consequence of striatal dopaminergic modulation. The second hypothesis was that the peripheral immune system dysfunctions were a consequence of neuroinflammatory processes. To study these hypotheses, the mutant *bapa* mouse was used as it is a validated experimental model of Kabuki syndrome. *Methods:* Exploratory behaviour, anxiety-like behaviour (light-dark test), repetitive/stereotyped behaviour (spontaneous and induced self-grooming), and tyrosine hydroxylase (TH), astrocyte glial fibrillary acidic protein (GFAP), and ionised calcium-binding adaptor molecule 1 (Iba1) striatal expressions were evaluated in female adult *bapa* and control mice. *Results:* Female *bapa* mice did not present anxiety-like behaviour, but exploratory hyperactivity and stereotyped behaviour both on the spontaneous and induced self-grooming tests. Striatal TH, GFAP, and Iba1 expressions were also increased in *bapa* mice. *Conclusion:* The exploratory hyperactivity and the stereotyped behaviour occurred in detriment of the striatal dopaminergic system hyperactivity and a permanent neuroinflammatory process.

Significant outcomes

- *Bapa* mice presented exploratory hyperactivity and stereotyped behaviour. Striatal tyrosine hydroxylase, glial fibrillary acidic protein, and ionised calcium-binding adaptor molecule 1 expressions were also increased in *bapa* mice.
- The behavioural phenotype was a result of the dopaminergic system hyperactivity.
- Dopaminergic system modulation was associated with a neuroinflammatory process.

Limitations

- Molecular studies about dopamine, tyrosine hydroxylase, cytokine levels, and the neuroinflammatory pathway.

Highlights

- *Bapa* mice presented exploratory hyperactivity and stereotyped behaviour. Striatal tyrosine hydroxylase, glial fibrillary acidic protein, and ionised calcium-binding adaptor molecule 1 expressions were also increased in *bapa* mice.
- The behavioural phenotype was a result of the dopaminergic system hyperactivity.
- Dopaminergic system modulation was associated with a neuroinflammatory process.

Introduction

Kabuki syndrome is a rare multisystem congenital disorder characterised by specific facial malformations (peculiar face with long or wide palpebral fissures, lower lateral eyelid eversion, arched eyebrows with the lateral third dispersed, prominent ears, depressed nasal tip, and

skeletal and dermatoglyphic abnormalities) that resemble the stage makeup used in Kabuki, a Japanese traditional theatrical form (Boniel *et al.*, 2021). Several other symptoms are documented, including poor physical growth, cardiac, gastrointestinal, and renal anomalies, motor and cognitive impairments, increased susceptibility to infections, and immune mediators' deficits (Van Laarhoven *et al.*, 2015; Wang *et al.*, 2019). Anxiety (Kalinousky *et al.*, 2022) and stereotyped behaviours (Sertcelik *et al.*, 2016; Boniel *et al.*, 2021) have been also reported in patients with Kabuki syndrome.

To study Kabuki syndrome and its behavioural, brain, immune, and genetic mechanisms, our group developed a mouse model: *bapa* mice. The *bapa* mouse model is a recessive mutant mouse also known as *bate palmas* (BALB/c^{bapa}). It arose from N-ethyl-N-nitrosourea mutagenesis (Massironi *et al.*, 2006). Genetic sequencing revealed the missense mutation NM_001033276: c.A3865G:p.T1289A in the lysine (K)-specific methyltransferase 2D (Kmt2d) gene on chromosome 15 (Yamamoto *et al.*, 2019). Indeed, mutations with a loss of function in the KMT2D gene in humans are mainly responsible for Kabuki syndrome (Ratbi *et al.*, 2013; Lu *et al.*, 2016). *Bapa* mice also present hyperactivity, sensory, and psychomotor impairments, such as hypotonia, and a slight motor coordination dysfunction (Yamamoto *et al.*, 2019; de Oliveira-Higa *et al.*, 2023). Some of these behavioural impairments, such as hyperactivity, are also found during the prepubertal period (Kirsten *et al.*, 2022). The behavioural findings were associated with dopaminergic system modulation, including increased gene expression of the D1 receptor (de Oliveira-Higa *et al.*, 2023) in adults and increased striatal tyrosine hydroxylase (TH) expression in juveniles (Kirsten *et al.*, 2022).

Besides our mouse model of Kabuki syndrome, there is also a model developed by Bjornsson and colleagues with a heterozygous deletion in the gene encoding the Kmt2d, leading to impairment of methyltransferase function: Kmt2d$^{+/βGeo}$ mice (Bjornsson *et al.*, 2014). This model is characterised by reduced neurogenesis, hippocampal memory defects (Bjornsson *et al.*, 2014), shortened long bones and ventral bowing of skulls associated with disrupted endochondral ossification (Fahrner *et al.*, 2019), and DNA methylation aberrations in peripheral blood (Goodman *et al.*, 2023). There are other studies in mice that are indirectly considered experimental models of the Kabuki syndrome. For example, loss of function of one allele for the KMT2A (Mll1) and Kmt2b (Mll2) genes is also related to cognitive, hippocampal, and neurogenesis defects (Kerimoglu *et al.*, 2013; Shen *et al.*, 2014; Jakovcevski *et al.*, 2015). There is currently no reasonable mechanism pointed out for the motor and immune deficits present in Kabuki syndrome patients.

Considering that most of the behavioural phenotype found in the Kabuki mouse model is associated with motor activity, the hypothesis of the present study is that behavioural impairments found in Kabuki syndrome are consequences of striatal dopaminergic modulation (Cerovic *et al.*, 2013; Prager and Plotkin, 2019). Moreover, the immune dysfunctions found in Kabuki syndrome may be in detriment of neuroinflammatory processes, since overactivity of astrocytes and microglia and other central nervous system components may affect susceptibility to infections and immune mediators expression (Schwab *et al.*, 2014; Ransohoff *et al.*, 2015; Edison, 2024). To evaluate these hypotheses, the *bapa* mouse model was used. Behavioural aspects of Kabuki syndrome were evaluated, such as anxiety, exploratory behaviour, and repetitive/stereotyped behaviours. TH, the rate-limiting enzyme of the dopamine biosynthesis pathway (Baker *et al.*, 2003), was

studied for the dopaminergic striatal modulation hypothesis. Astrocytic glial fibrillary acidic protein (GFAP, the major protein constituent of glial intermediate filaments in differentiated astrocytes of the central nervous system) (Sofroniew and Vinters, 2010) and ionised calcium-binding adaptor molecule 1 (Iba1, a macrophage/microglial marker) (Kempuraj *et al.*, 2024) were studied for the neuroinflammatory hypothesis.

Material and methods

Ethics statement

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

The present study was carried out in strict accordance with the recommendations of the ARRIVE guidelines and the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Paulista University, Brazil (Permit Number: 035/17). All efforts were made to minimise suffering, reduce the number of animals used, and utilise alternatives to *in vivo* techniques when available. The experiments were also performed in accordance with good laboratory practice protocols and quality assurance methods.

Animals, groups, and experimental design

Female adult BALB/c and BALB/c^{bapa} (*bapa*) mice (*Mus musculus*) with approximately 100 days of age on the luteal phase of the oestrous cycle were used (n = 7/group). The luteal phase was confirmed through microscopic analysis of histological sections from the ovary and uterine horns (Akinjiola *et al.*, 2018). Mice were obtained from the Institute of Biomedical Sciences (University of São Paulo, São Paulo, Brazil) and housed at Paulista University (São Paulo, Brazil) under standard conditions. The mice housing, nutritional conditions, and daily handling and care were standard and previously described by our group (Kirsten *et al.*, 2022).

Mice were first evaluated in the light-dark test and then for spontaneous self-grooming behaviour. Twenty-four hours later, they were evaluated in the splash test, and immediately, their brains were processed for immunohistochemical analysis (TH, GFAP, and Iba1). All the experiments were performed between 9:00 and 11:00 AM to minimise the effects of circadian rhythms. All behavioural assays and the immunohistochemical analyses were performed by investigators who were blinded to the treatment groups.

Light-dark test

The light-dark test was performed to evaluate anxiety-like behaviour and exploratory behaviour as previously described (Kirsten *et al.*, 2020). This model is based on the inherent conflict between the exploratory drive to a novel place and the avoidance of a lit compartment (Campos *et al.*, 2013). The apparatus consisted of an acrylic box (45 × 27 × 20 cm) containing two compartments (separated by a door with 9 × 7 cm): dark room with black walls and floor (17 cm), and a light room, with white walls and floor (26 cm) and illuminated with a white fluorescent lamp

(15W, 4100K). Each mouse was individually placed in the centre of the light room, facing the wall opposite the door. The following parameters were evaluated over a period of 5 min: dark side entry latency (s), total times (s) spent in the dark and in the light sides, and total rearing frequency. The testing room, which was isolated from the experimenter, was a small room with dim lighting. A video camera mounted above the arena was used to collect the data. The apparatus was washed with a 5% alcohol/water solution before placement of the animals to obviate possible biasing effects from odour cues left by previous mice.

Spontaneous self-grooming behaviour

Spontaneous self-grooming behaviour was evaluated immediately after a 30-min habituation period, conducted immediately after the light-dark test. Each mouse was individually placed in the centre of a clear observation cage (30 × 16 × 19 cm), and after the habituation period, the following parameters were evaluated over a period of 30 min: head washing, body grooming, paw/leg licking, and tail/genital grooming total times (s) (Kirsten and Bernardi, 2017). The testing room and cleaning procedures were the same as those used for the light-dark test. A video camera mounted in front of the arena was used to collect the data.

Splash test

Twenty-four hours after the evaluation of the spontaneous self-grooming behaviour, the splash test was conducted in the same clear observation cage, according to a previous study (Reis-Silva *et al.*, 2019). The splash test evaluated the induced self-grooming after spraying a 10% sucrose solution on the dorsal coat of each mouse. The same parameters evaluated for the spontaneous self-grooming behaviour were evaluated over a period of 5 min: head washing, body grooming, paw/leg licking, and tail/genital grooming total times (s). The testing room, video recording, and cleaning procedures were the same as those used for the spontaneous self-grooming evaluation.

TH, GFAP, and Iba1 analyses

Immediately after behavioural tests, mice were euthanised (anaesthetic overdose, thiopental, 200 mg/kg, i.p.), and their brains were collected and fixed in 10% buffered formalin for 72 h for usual histological procedures. The striatum was studied for the immunohistochemical expression of TH, GFAP, and Iba1 proteins (Guimaraes Marques *et al.*, 2019). It is considered a motor system brain area, involved in the modulation of movements, emotions, and cognition (Groenewegen, 2003), and its relation with neuroinflammation (Abg Abd Wahab *et al.*, 2019; Mancini *et al.*, 2021). The striatum was processed for immunohistochemical analysis as previously described (Kirsten *et al.*, 2022). Briefly, four sections (5 μm thick) per rat were made from each striatum. Immunohistochemistry was performed using the chain polymer-conjugated staining method. Monoclonal anti-TH antibody (1:1000, Millipore Cat# IHCR1005-6, Chemicon IHC Select Research, Germany), polyclonal rabbit anti-GFAP immunoglobulin (1:100, Agilent Cat# Z0334, Santa Clara, CA, USA), and polyclonal rabbit anti-Iba1 immunoglobulin (1:100; GeneTex Cat# GTX101495, Irvine, CA, USA) were used as primary antibodies according to protocol from the suppliers, followed by the EnVision+ Kit for detection (Agilent Cat# K4011; HRP, Rabbit, DAB+, Santa Clara, CA, USA). Antigen retrieval was achieved by heating the slides in citrate buffer (pH 6.0) at 95ºC for

15 min in a steamer. PBS solution instead of the primary antibody was used as the negative control during immunohistochemical staining. The sections were counterstained with Harris haematoxylin and mounted with DPX (06522, Sigma Aldrich, St Louis, MO, USA). From each individual immunostained section (for TH, GFAP, and Iba1), 5–6 photomicrographs were taken (40× objective, Nikon E200 microscope, equipped with a Nikon Coolpix digital camera linked to a liquid crystal display monitor, Kanagawa, Japan). Morphometric analysis was performed using the Image Pro-Plus 6 software (Media Cybernetics, Rockville, MD, USA), calibrated with digital colour filters regulating red, green, and blue bits, in such a way that only immunostained cells were included and the background staining was excluded from the measurement. For TH, GFAP, and Iba1 immunostaining quantification, we used the index per area to represent the extent of the area immunostained for TH in neurones, GFAP in astrocytes or Iba1 in microglial cells compared to the total area of the image (being zero, as the complete absence of staining, and one, as the total staining of the area). For statistical purposes, the means of 5–6 photomicrograph values from each mouse were used as units.

Statistical analysis

Normality was verified using Shapiro–Wilk or Kolmogorov–Smirnov tests, depending on the sample size (alpha = 0.05). When necessary, an outlier identification test was applied (ROUT, $Q = 5\%$). The Student's *t*-test (unpaired, two-tailed) was used to compare the parametric data between the groups. The Mann–Whitney *U*-test was used to compare the nonparametric data between the groups. The results are expressed as mean ± SEM in box and whiskers (min to max, showing all values) graphs. In all cases, the results were considered significant if $p < 0.05$.

Results

In the light-dark test, both groups passed the normality tests for all the evaluated parameters (Supplementary Table 1). None of the anxiety parameters was altered between groups: dark side entry latency (Fig. 1A), time spent in the dark side (Fig. 1B), and time spent in the light side (Fig. 1C). However, female *bapa* mice presented increased rearing behaviour, compared with BALB/c data (Fig. 1D). In other words, *bapa* mice did not present anxiety-like behaviour but increased exploratory behaviour.

In the spontaneous self-grooming behaviour evaluation, both groups passed the normality tests for all the evaluated parameters (Supplementary Table 1). All the evaluated parameters were altered between groups. Specifically, compared with BALB/c data, female *bapa* mice presented increased time spent for head washing (Fig. 2A), body grooming (Fig. 2B), paw/leg licking (Fig. 2C), and tail/genital grooming (Fig. 2D). Therefore, *bapa* mice presented increased spontaneous self-grooming behaviour.

In the splash test, one of the four parameters did not pass the normality tests for both groups: tail/genital grooming time (Supplementary Table 1). Compared with BALB/c data, female *bapa* mice presented increased time spent for body grooming (Fig. 2F) and paw/leg licking (Fig. 2G), but not for head washing (Fig. 2E) and tail/genital grooming (Fig. 2H). Therefore, *bapa* mice presented increased induced self-grooming behaviour in the splash test.

For the immunohistochemical analysis of TH expression, both groups passed the normality tests (Supplementary Table 1). Female
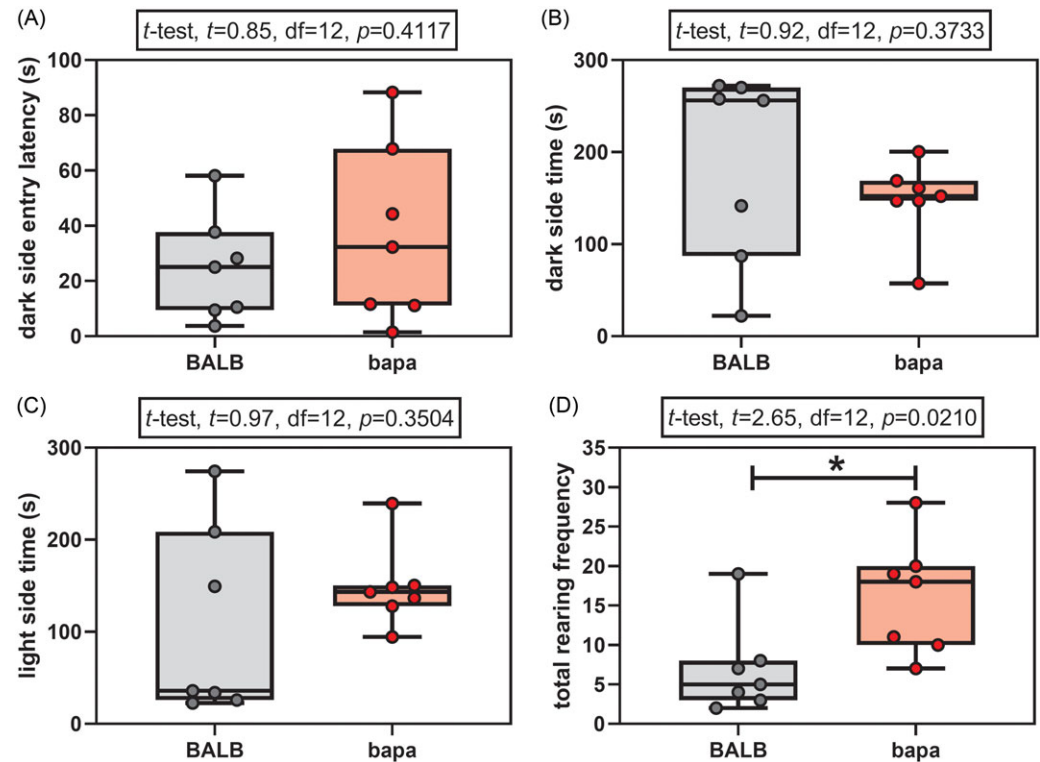
Figure 1. Light-dark test. Light-dark test of adult BALB/c and BALB/c^{bapa} (*bapa*) mice (n = 7 mice per group). *p < 0.05 (Student's *t*-test). Data are expressed as mean ± SEM in box and whiskers (min to max, showing all values) graphs.

bapa mice presented increased striatal TH expression, compared with BALB/c data (Fig. 3A–C).

For the immunohistochemical analysis of GFAP expression, both groups passed the normality tests (Supplementary Table 1). Female *bapa* mice presented increased striatal GFAP expression, compared with BALB/c data (Fig. 3D–F).

For the immunohistochemical analysis of Iba1 expression, both groups passed the normality tests (Supplementary Table 1). Female *bapa* mice presented increased striatal Iba1 expression, compared with BALB/c data (Fig. 3G–I).

Discussion

Although anxiety is not considered a core symptom of Kabuki syndrome (Van Laarhoven *et al.*, 2015; Wang *et al.*, 2019), there is tangible evidence that it should be considered a neurobehavioral feature: a cohort of 60 individuals with molecularly confirmed Kabuki syndrome presents higher anxiety scores than controls (Kalinousky *et al.*, 2022). Moreover, patients with a mosaic KMT2D variant were described with similar systemic features and anxiety manifestations (Boniel *et al.*, 2021). Thus, the present study evaluated anxiety-like behaviour in the *bapa* mouse model. The light-dark test was chosen for the evaluation of anxiety-like behaviour since it is considered a reliable and popular test for this purpose on mice (Bourin and Hascoet, 2003; Campos *et al.*, 2013).

Presently, none of the evaluated anxiety parameters were affected; that is, female adult *bapa* mice did not present anxiety-like behaviour. Similarly, prepubertal and pubertal *bapa* mice do not present anxiety-like behaviour evaluated on the open-field test (Kirsten *et al.*, 2022). Adult *bapa* mice also do not present anxiety-like behaviour evaluated on the elevated plus maze test (Oliveira, 2017). Therefore, although anxiety is a secondary symptom of Kabuki syndrome, the *bapa* mouse model consistently did not result in anxiety-like behaviour.

However, female *bapa* mice presented increased exploratory behaviour demonstrated by increased rearing frequency in the light-dark test. This pattern was previously demonstrated in the *bapa* mouse model using different variables: adult males and females in the open-field test (increased distance travelled, average speed, and rearing) (Yamamoto *et al.*, 2019); adult males in the open-field test observed for four consecutive days (increased locomotion and rearing and decreased immobility) (de Oliveira-Higa *et al.*, 2023); and prepubertal period in the open-field test (increased rearing frequency) (Kirsten *et al.*, 2022), always comparing to their controls. Thereby, *bapa* mice presented locomotory hyperactivity, a symptom described in patients with Kabuki syndrome (Mervis *et al.*, 2005; Sertcelik *et al.*, 2016).

To understand the neurobiological mechanism responsible for the locomotory hyperactivity, striatal TH expression was evaluated. Female adult *bapa* mice presented increased striatal TH expression, indicating increased dopamine synthesis in the striatal dopaminergic system (Baker *et al.*, 2003). A similar result was found in the prepubertal period (Kirsten *et al.*, 2022). Additionally, adult male *bapa* mice present increased gene expression of the striatal D1 receptor (de Oliveira-Higa *et al.*, 2023). Taken together, the behavioural findings were associated with dopaminergic system modulation; that is, *bapa* mice presented striatal dopaminergic system hyperactivity, which triggered an increased exploratory activity.

Self-grooming of mice was evaluated because it is a behavioural tool used for studies about stereotyped behaviour (Crawley, 2012), which is reported in some patients with Kabuki syndrome (Sertcelik *et al.*, 2016; Boniel *et al.*, 2021). Self-grooming is considered a behavioural pattern very sensitive to genetic manipulations and to study genotype differences among selected mouse strains (Kalueff *et al.*, 2007) and human neurological disorders (Kalueff *et al.*, 2016). Therefore, the self-grooming behavioural study was applied for the understanding of the mutant mouse *bapa* and the Kabuki syndrome.
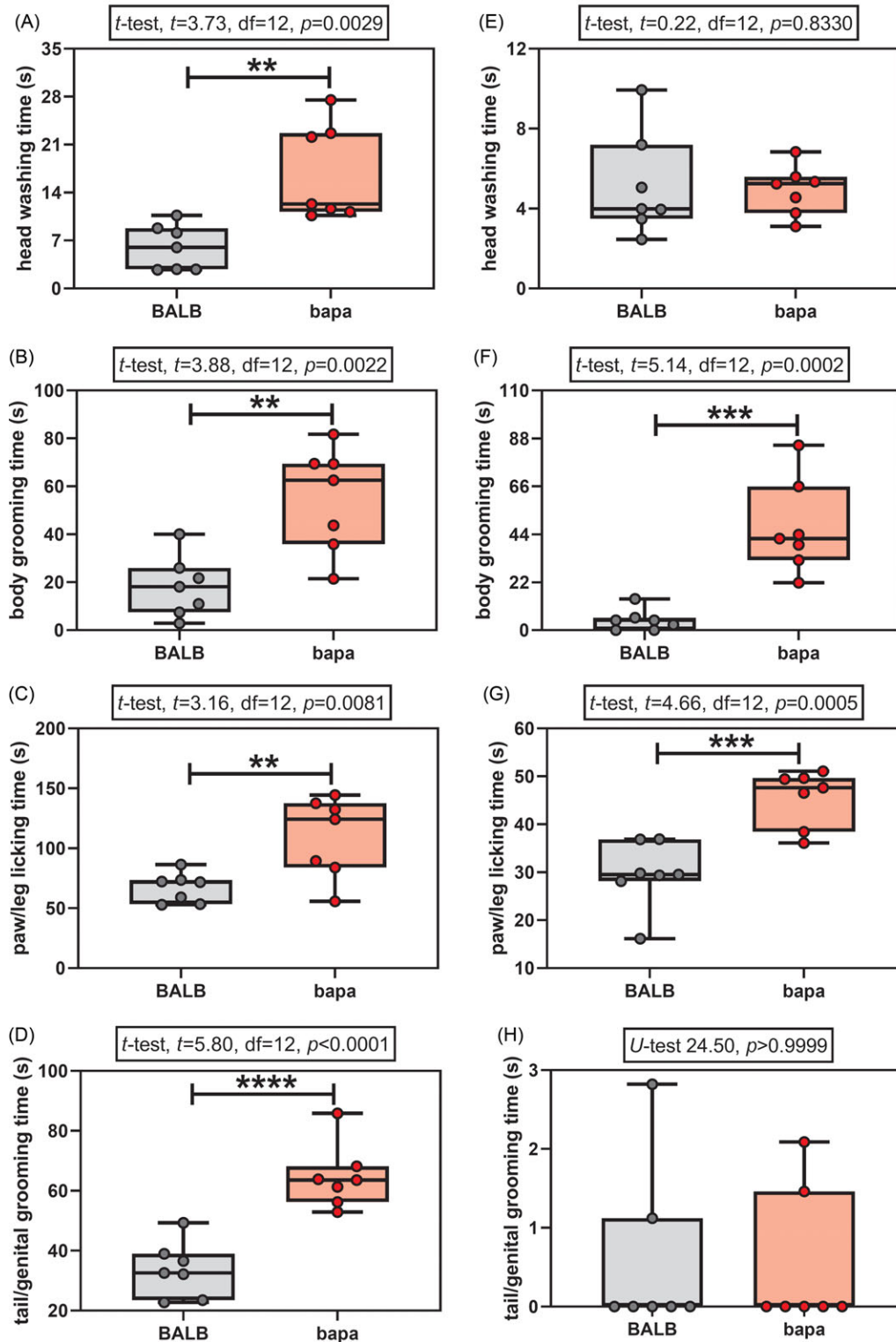
Figure 2. Self-grooming behaviour. Spontaneous and induced (splash test) self-grooming behaviour of adult BALB/c and BALB/c^{bapa} (*bapa*) mice (n = 7 mice per group). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; and ****$p < 0.0001$ (Student's *t*-test and Mann–Whitney *U*-test). Data are expressed as mean ± SEM in box and whiskers (min to max, showing all values) graphs.

A typical grooming chain in rodents is embedded into different predictable patterns and microstructures, which include head washing, body grooming, paw/leg licking, and tail/genital grooming (Kalueff *et al.*, 2007). Simply assessing the 'amount' of animal total grooming may be insufficient for correct data interpretation and analysis (Kalueff *et al.*, 2007). Self-grooming can be elicited by various environmental conditions, such as under basal conditions, after an acclimation period in the recording chamber and induced artificially, for example, following misting rodents with water (using spray) (Kalueff *et al.*, 2007). The present study evaluated
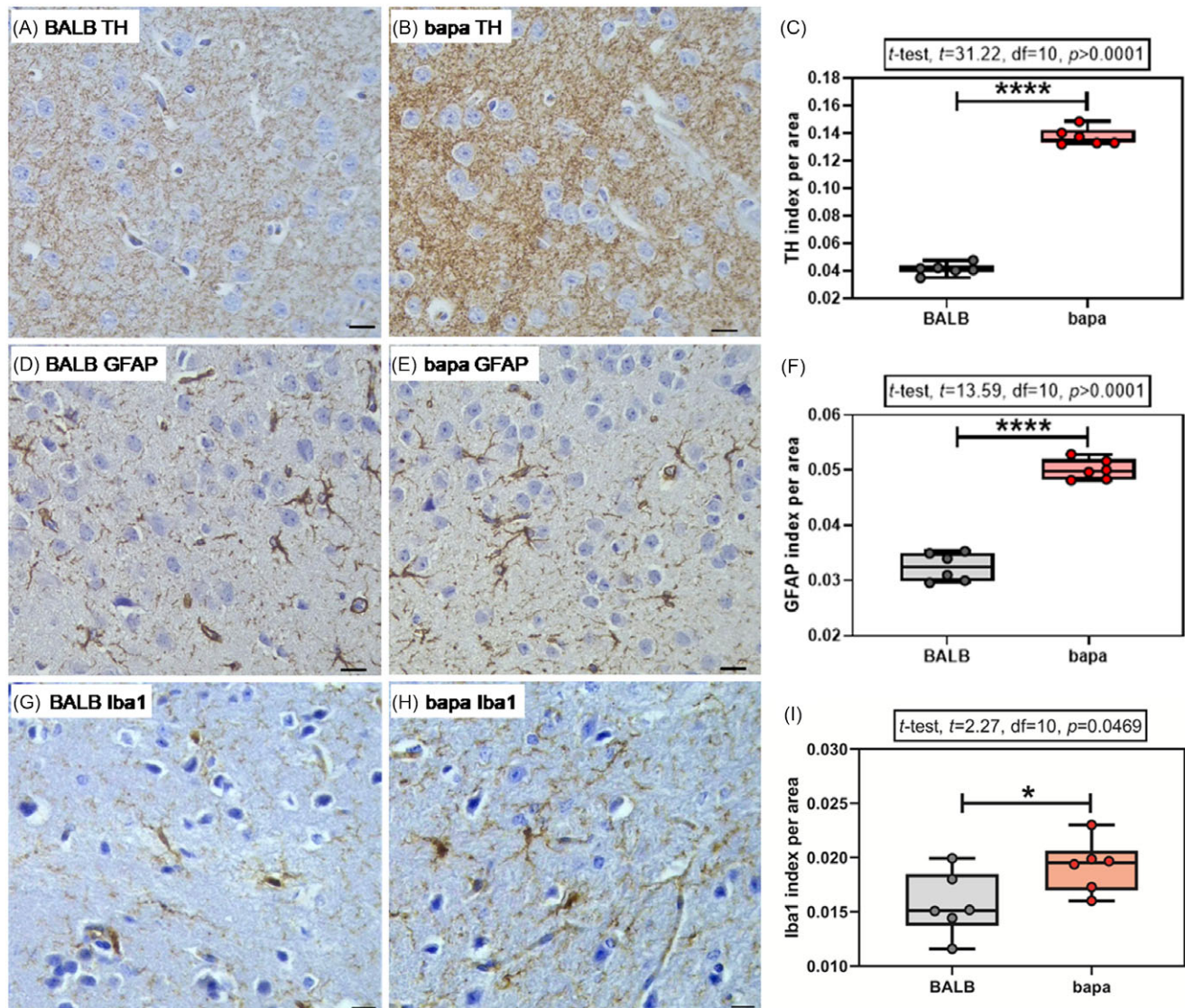
Figure 3. TH, GFAP, and Iba1. (A–C) Tyrosine hydroxylase (TH), (D–F) astrocyte glial fibrillary acidic protein (GFAP), and (G-I) microglial Iba1 expressions in the striatum of adult BALB/c and BALB/c^{bapa} (*bapa*) mice (n = 6 mice per group; 5–6 photomicrographs from each individual brain section). Morphometric analysis from TH, GFAP, and Iba1-immunolabelled sections. Scale bar = 50 μm. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; and ****$p < 0.0001$ (Student's *t*-test). Data are expressed as mean ± SEM in box and whiskers (min to max, showing all values) graphs.

these two environmental conditions: basal for the spontaneous grooming and the elicited grooming (using a spray – splash test). Both models revealed increased self-grooming in the female *bapa* mice, compared with the controls. However, the spontaneous self-grooming test resulted in more expressive differences than the splash test. Taken together with its natural conception and a less stressful protocol, the spontaneous self-grooming test was considered a reliable test to study the mutant mouse *bapa* and the Kabuki syndrome.

Self-grooming sequencing, chain initiation, and chain completion in rodents are strongly bidirectionally affected by striatal dopaminergic system modulation, including lesions of the dopamine-containing nigrostriatal tract, administration of various dopaminergic drugs, genetic mutations, and psychological stress (Cromwell and Berridge, 1996; Burguiere *et al.*, 2013; Kalueff *et al.*, 2016). For example, dopamine D1 receptor activation by D1 agonists induces excessive grooming (Berridge and Aldridge, 2000).

Similarly, transgenic mice with reduced TH immunoreactivity in different brain areas present impaired grooming behaviour (Aloe and Fiore, 1997). Thus, the increased TH expression in the striatum of *bapa* mice was hypothesised as the explanation of the pathway responsible for the increased self-grooming and stereotyped behaviour. However, further molecular studies should be conducted to reveal if this is the only pathway involved and to deepen the knowledge.

As previously reported, Kabuki syndrome patients are commonly diagnosed with immune system deficiencies. It is described as a lack of antibodies, loss of memory cells, IgA deficiency, hyper-IgM syndrome, disturbed differentiation of terminal B-cells (humoral immunodeficiency), and some autoimmune diseases (Van Laarhoven *et al.*, 2015; Wang *et al.*, 2019). The hypothesis of the present study was that this immune dysfunction may be in detriment of neuroinflammation, since overactivity of astrocytes and microglia, as well as other central

nervous system components may affect susceptibility to infections and immune mediators' expression (Schwab *et al.*, 2014; Ransohoff *et al.*, 2015; Edison, 2024). As Kabuki syndrome is characterised by immune dysregulation, up to 17% of its patients present immune thrombocytopenia, often associated with other hematological autoimmune diseases, including autoimmune haemolytic anaemia, eventually resulting in Evans disease, with recurrent respiratory diseases and chronic lung inflammation (Leonardi *et al.*, 2023). In fact, *bapa* mice presented increased striatal GFAP and Iba1 expressions. Clearing of dopamine excess may also explain astrocyte hyperactivity since these cells perform metabolic, structural, homeostatic, and neuroprotective functions (Sofroniew and Vinters, 2010). It is recognised that astrocytes from the striatum and cortex express D1-like (D1 and D5) and D2-like receptors (D2, D3, and D4) and dopamine signalling impacts on astrocyte morphology and gene expression (Corkrum and Araque, 2021). Inflammation induced by activated microglia can directly damage dopaminergic neurones, inhibiting dopamine synthesis, reuptake, and receptor activity (She *et al.*, 2024). Thereby, the increased expression of striatal GFAP and Iba1 revealed a neuroinflammatory process, which was hypothesised as the responsible for the immune dysfunction found in the Kabuki syndrome.

It is important to mention that the increased striatal GFAP and Iba1 expressions were observed in female adult *bapa* mice not challenged with any immunological agent, which reassembled not an acute, but a permanent (chronic) striatal neuroinflammatory process. This is a novel result, considering that the previous study of our group revealed increased GFAP expression in juveniles after an LPS challenge (Kirsten *et al.*, 2022).

Incidentally, a persistent acute neuroinflammation can turn to a chronic neuroinflammation as it accumulates damage, resulting in neuronal degeneration (Abg Abd Wahab *et al.*, 2019). Neuroinflammation is the response of the central nervous system to disturbed homeostasis and typifies several neurological and neurodegenerative diseases, such as Parkinson's disease, Huntington's disease, multiple sclerosis, narcolepsy, and autism (Ransohoff *et al.*, 2015; Abg Abd Wahab *et al.*, 2019; Mancini *et al.*, 2021). Specifically, astroglial-mediated inflammation plays a prominent role in the pathogenesis of neurodegenerative diseases, such as dementia and Alzheimer's disease (Edison, 2024). In fact, intracellular signalling pathways are completely controlled by astrocytes during inflammation. Astrocytes and microglia are involved in cellular and molecular functions for degeneration, vascular signalling, and glial–neuronal interactions (Abg Abd Wahab *et al.*, 2019).

Besides the explanation of the peripheral immunological deficits found in the Kabuki syndrome, striatal neuroinflammation could also explain the striatal dopaminergic system hyperactivity (behaviour and TH expression) presently found. There is robust evidence of the cross talk between neurotransmitters and neuroinflammation in the striatum in the mediation of motor behaviour (Abg Abd Wahab *et al.*, 2019; Mancini *et al.*, 2021). For example, neuroinflammation is found to be involved in the alterations in dopamine neurotransmission, whereby cytokines ultimately lead to decreased dopamine synthesis, thus decreasing dopamine function, which could lead to neurodegeneration (Abg Abd Wahab *et al.*, 2019). Apropos, dopamine can act as both an inhibitory and excitatory neurotransmitter depending upon its location in the brain and which receptor it binds to (Nakamura *et al.*, 2014). Moreover, the striatum acts as one of the main target regions for

dopamine involving the regulation of motor functions (Abg Abd Wahab *et al.*, 2019). Therefore, it is plausible that the striatal dopaminergic system hyperactivity in the *bapa* mice is a consequence of a permanent striatal neuroinflammatory process.

The present study evaluated only female mice based on our previous studies. The motor/exploratory hyperactivity of adult *bapa* mice found in the behavioural assessments (increased distance travelled, average speed, and rearing) presents the same statistical difference, comparing male and female *bapa* mice with their respective controls (Yamamoto *et al.*, 2019). The same scenario is revealed in the behavioural and brain analyses of prepubertal and pubertal mice (Kirsten *et al.*, 2022). Therefore, considering that there are no sex-specific effects in the behavioural and brain tests of *bapa* mice, the effort to minimise the number of animals used in the experiments, and the tendency in neuroscience studies to avoid sex-discrimination (comparing males and females), only female mice were studied in the present study

In conclusion, *bapa* mice did not present anxiety-like behaviour but exploratory hyperactivity and stereotyped behaviour. This phenotype occurred in detriment of the striatal dopaminergic system hyperactivity and a permanent neuroinflammatory process.

References

Abg Abd Wahab DY, Gau CH, Zakaria R, Muthu Karuppan MK, Bs AR, Abdullah Z, Alrafiah A, Abdullah JM and Muthuraju S (2019) Review on cross talk between neurotransmitters and neuroinflammation in striatum and cerebellum in the mediation of motor behaviour. *Biomed Research International* **2019**, 1767203–10.

Akinjiola AM, Ajala OO, Aina OO, Oyebanji VO and Olukunle JO (2018) Histomorphometry and protein expression from the ovary and uterine horns of Wistar strain albino rats treated with methanol leave extract of Parquetina nigrescens. *Drug Research* **68**(12), 717–724.

Aloe L and Fiore M (1997) TNF-alpha expressed in the brain of transgenic mice lowers central tyroxine hydroxylase immunoreactivity and alters grooming behavior. *Neuroscience Letters* **238**(1-2), 65–68.

Baker H, Kobayashi K, Okano H and Saino-Saito S (2003) Cortical and striatal expression of tyrosine hydroxylase mRNA in neonatal and adult mice. *Cellular and Molecular Neurobiology* **23**(4/5), 507–518.

Berridge KC and Aldridge JW (2000) Super-stereotypy I: enhancement of a complex movement sequence by systemic dopamine D1 agonists. *Synapse* **37**(3), 194–204.

Bjornsson HT, Benjamin JS, Zhang L, Weissman J, Gerber EE, Chen YC, Vaurio RG, Potter MC, Hansen KD and Dietz HC (2014) Histone deacetylase inhibition rescues structural and functional brain deficits in a mouse model of Kabuki syndrome. *Science Translational Medicine* **6**(256), 256ra135.

Boniel S, Szymanska K, Smigiel R and Szczaluba K (2021) Kabuki syndrome-clinical review with molecular aspects. *Genes (Basel)* **12**(4), 468.

Bourin M and Hascoet M (2003) The mouse light/dark box test. *European Journal of Pharmacology* **463**(1-3), 55–65.

Burguiere E, Monteiro P, Feng G and Graybiel AM (2013) Optogenetic stimulation of lateral orbitofronto-striatal pathway suppresses compulsive behaviors. *Science* **340**(6137), 1243–1246.

Campos AC, Fogaca MV, Aguiar DC and Guimaraes FS (2013) Animal models of anxiety disorders and stress. *Revista Brasileira de Psiquiatria*, 35 Suppl 2, S101–11.

Cerovic M, D'isa R, Tonini R and Brambilla R (2013) Molecular and cellular mechanisms of dopamine-mediated behavioral plasticity in the striatum. *Neurobiology of Learning and Memory* **105**, 63–80.

Corkrum M and Araque A (2021) Astrocyte-neuron signaling in the mesolimbic dopamine system: the hidden stars of dopamine signaling. *Neuropsychopharmacology* **46**(11), 1864–1872.

Crawley JN (2012) Translational animal models of autism and neuro-developmental disorders. *Dialogues Clin Neurosci* **14**(3), 293–305.

Cromwell HC and Berridge KC (1996) Implementation of action sequences by a neostriatal site: a lesion mapping study of grooming syntax. *Journal of Neuroscience* **16**(10), 3444–3458.

De Oliveira-Higa MA, Da Silva Rodrigues P, Sampaio ACS, De Camargo Coque A, Kirsten TB, Massironi SMG, Alexandre-Ribeiro SR, Mori CMC, Da Silva RA and Bernardi MM (2023) The dopaminergic D1 receptor modulates the hyperactivity of bapa mutant mice. *Behavioural Brain Research* **452**, 114562.

Edison P (2024) Astroglial activation: current concepts and future directions. *Alzheimers & Dementia* **20**(4), 3034–3053.

Fahrner JA, Lin WY, Riddle RC, Boukas L, Deleon VB, Chopra S, Lad SE, Luperchio TR, Hansen KD and Bjornsson HT (2019) Precocious chondrocyte differentiation disrupts skeletal growth in Kabuki syndrome mice. *JCI Insight* **4**(20), e129380.

Goodman SJ, Luperchio TR, Ellegood J, Chater-Diehl E, Lerch JP, Bjornsson HT and Weksberg R (2023) Peripheral blood DNA methylation and neuroanatomical responses to HDACi treatment that rescues neurological deficits in a Kabuki syndrome mouse model. *Clinical Epigenetics* **15**(1), 172.

Groenewegen HJ (2003) The basal ganglia and motor control. *Neural Plasticity* **10**(1-2), 107–120.

Guimaraes Marques MJ, Real CC, Victorino DB, Britto LR, Cavalheiro EA, Scorza FA, Ferraz HB and Scorza CA (2019) Endogenous protection against the 6-OHDA model of Parkinson's disease in the amazonian rodent proechimys. *Neuroscience Letters* **709**, 134381.

Jakovcevski M, Ruan H, Shen EY, Dincer A, Javidfar B, Ma Q, Peter CJ, Cheung I, Mitchell AC, Jiang Y, Lin CL, Pothula V, Stewart AF, Ernst P, Yao WD and Akbarian S (2015) Neuronal Kmt2a/Mll1 histone methyltransferase is essential for prefrontal synaptic plasticity and working memory. *Journal of Neuroscience* **35**(13), 5097–5108.

Kalinousky AJ, Rapp T, Hijazi H, Johnson J, Bjornsson HT and Harris JR (2022) Neurobehavioral phenotype of Kabuki syndrome: anxiety is a common feature. *Frontiers in Genetics* **13**, 1007046.

Kalueff AV, Aldridge JW, Laporte JL, Murphy DL and Tuohimaa P (2007) Analyzing grooming microstructure in neurobehavioral experiments. *Nature Protocols* **2**(10), 2538–2544.

Kalueff AV, Stewart AM, Song C, Berridge KC, Graybiel AM and Fentress JC (2016) Neurobiology of rodent self-grooming and its value for translational neuroscience. *Nature Reviews Neuroscience* **17**(1), 45–59.

Kempuraj D, Dourvetakis KD, Cohen J, Valladares DS, Joshi RS, Kothuru SP, Anderson T, Chinnappan B, Cheema AK, Klimas NG and

Theoharides TC (2024) Neurovascular unit, neuroinflammation and neurodegeneration markers in brain disorders. *Frontiers in Cellular Neuroscience* **18**, 1491952.

Kerimoglu C, Agis-Balboa RC, Kranz A, Stilling R, Bahari-Javan S, Benito-Garagorri E, Halder R, Burkhardt S, Stewart AF and Fischer A (2013) Histone-methyltransferase MLL2 (KMT2B) is required for memory formation in mice. *Journal of Neuroscience* **33**(8), 3452–3464.

Kirsten TB and Bernardi MM (2017) Prenatal lipopolysaccharide induces hypothalamic dopaminergic hypoactivity and autistic-like behaviors: repetitive self-grooming and stereotypies. *Behavioural Brain Research* **331**, 25–29.

Kirsten TB, Cabral D, Galvao MC, Monteiro R, Bondan EF and Bernardi MM (2020) Zinc, but not paracetamol, prevents depressive-like behavior and sickness behavior, and inhibits interferon-gamma and astrogliosis in rats. *Brain Behavior and Immunity* **87**, 489–497.

Kirsten TB, Silva EP, Biondi TF, Rodrigues PS, Cardoso CV, Massironi SMG, Mori CMC, Bondan EF and Bernardi MM (2022) Bate palmas mutant mice as a model of Kabuki syndrome: higher susceptibility to infections and vocalization impairments? *Journal of Neuroscience Research* **100**(7), 1438–1451.

Leonardi L, Testa A, Feleppa M, Paparella R, Conti F, Marzollo A, Spalice A, Giona F, Gnazzo M, Andreoli GM, Costantino F and Tarani L (2023) Immune dysregulation in Kabuki syndrome: a case report of evans syndrome and hypogammaglobulinemia. *Frontiers in Pediatrics* **11**, 1087002.

Lu J, Mo G, Ling Y and Ji L (2016) A novel KMT2D mutation resulting in Kabuki syndrome: a case report. *Molecular Medicine Reports* **14**(4), 3641–3645.

Mancini A, Ghiglieri V, Parnetti L, Calabresi P and Di Filippo M (2021) Neuro-immune cross-talk in the striatum: from basal ganglia physiology to circuit dysfunction. *Frontiers in Immunology* **12**, 644294.

Massironi SM, Reis BL, Carneiro JG, Barbosa LB, Ariza CB, Santos GC, Guenet JL and Godard AL (2006) Inducing mutations in the mouse genome with the chemical mutagen ethylnitrosourea. *Brazilian Journal of Medical and Biological Research* **39**(9), 1217–1226.

Mervis CB, Becerra AM, Rowe ML, Hersh JH and Morris CA (2005) Intellectual abilities and adaptive behavior of children and adolescents with Kabuki syndrome: a preliminary study. *American Journal of Medical Genetics Part A* **132A**(3), 248–255.

Nakamura T, Sato A, Kitsukawa T, Momiyama T, Yamamori T and Sasaoka T (2014) Distinct motor impairments of dopamine D1 and D2 receptor knockout mice revealed by three types of motor behavior. *Frontiers in Integrative Neuroscience* **8**, 56.

Oliveira MA (2017) Hiperatividade do sistema dopaminérgico central em camundongo mutante bapa: um modelo experimental da síndrome de Kabuki. Universidade Paulista: Mestrado.

Prager EM and Plotkin JL (2019) Compartmental function and modulation of the striatum. *Journal of Neuroscience Research* **97**(12), 1503–1514.

Ransohoff RM, Schafer D, Vincent A, Blachere NE and Bar-Or A (2015) Neuroinflammation: ways in which the immune system affects the brain. *Neurotherapeutics* **12**(4), 896–909.

Ratbi I, Fejjal N, Micale L, Augello B, Fusco C, Lyahyai J, Merla G and Sefiani A (2013) Report of the first clinical case of a Moroccan Kabuki patient with a novel MLL2 mutation. *Molecular Syndromology* **4**(3), 152–156.

Reis-Silva TM, Sandini TM, Calefi AS, Orlando BCG, Moreira N, Lima APN, Florio JC, Queiroz-Hazarbassanov NGT and Bernardi MM (2019) Stress resilience evidenced by grooming behaviour and dopamine levels in male mice selected for high and low immobility using the tail suspension test. *European Journal of Neuroscience* **50**(6), 2942–2954.

Schwab JM, Zhang Y, Kopp MA, Brommer B and Popovich PG (2014) The paradox of chronic neuroinflammation, systemic immune suppression, autoimmunity after traumatic chronic spinal cord injury. *Experimental Neurology* **258**, 121–129.

Sertcelik M, Ugur C, Sahin Akozel A and Gurkan CK (2016) A child with Kabuki syndrome and autism spectrum disorder. *Noro Psikiyatri Arsivi* **53**(3), 280–282.

She K, Yuan N, Huang M, Zhu W, Tang M, Ma Q and Chen J (2024) Emerging role of microglia in the developing dopaminergic system: perturbation by early life stress. *Neural Regeneration Research* **21**(1), 126–140.

Shen E, Shulha H, Weng Z and Akbarian S (2014) Regulation of histone H3K4 methylation in brain development and disease. *Philosophical Transactions of the Royal Society of London B Biological Sciences* **369**(1652), 20130514.

Sofroniew MV and Vinters HV (2010) Astrocytes: biology and pathology. *Acta Neuropathologica* **119**(1), 7–35.

Van Laarhoven PM, Neitzel LR, Quintana AM, Geiger EA, Zackai EH, Clouthier DE, Artinger KB, Ming JE and Shaikh TH (2015) Kabuki syndrome genes KMT2D and KDM6A: functional analyses demonstrate critical roles in craniofacial, heart and brain development. *Human Molecular Genetics* **24**(15), 4443–4453.

Wang YR, Xu NX, Wang J and Wang XM (2019) Kabuki syndrome: review of the clinical features, diagnosis and epigenetic mechanisms. *World Journal of Pediatrics* **15**(6), 528–535.

Yamamoto PK, Souza TA, Antiorio ATFB, Zanatto DA, Garcia-Gomes MDSA, Alexandre-Ribeiro SR, Oliveira NDS, Menck CFM, Bernardi MM, Massironi SMG and Mori CMC (2019) Genetic and behavioral characterization of a Kmt2d mouse mutant, a new model for Kabuki syndrome. *Genes, Brain and Behavior* **18**(8). e12568.