






## ORIGINAL ARTICLE

# A cross-sectional, prospective ocular motor study in 72 patients with Niemann-Pick disease type C

Tatiana Bremova-Ertl<sup>1,2</sup>  | Larry Abel<sup>3</sup>  | Mark Walterfang<sup>4,5</sup>  | Ettore Salsano<sup>6</sup>  | Anna Ardisson<sup>6</sup>  | Věra Malinová<sup>7</sup> | Miriam Kolníková<sup>8</sup> | Jordi Gascón Bayarri<sup>9</sup> | Ali Reza Tavasoli<sup>10</sup> | Mahmoud Reza Ashrafi<sup>10</sup> | Yasmina Amraoui<sup>11</sup> | Eugen Mengel<sup>11</sup> | Stefan A. Kolb<sup>12</sup> | Andreas Brecht<sup>12</sup> | Stanislavs Bardins<sup>13</sup> | Michael Strupp<sup>13</sup>

<sup>1</sup>Department of Neurology, University Hospital Bern (Inselspital) and University of Bern, Bern, Switzerland

<sup>2</sup>Center for Rare Diseases, University Hospital Bern (Inselspital) and University of Bern, Bern, Switzerland

<sup>3</sup>Optometry & Vision Science, School of Medicine, Deakin University, Waurn Ponds, Victoria, Australia

<sup>4</sup>Neuropsychiatry Unit, Royal Melbourne Hospital, Parkville, Victoria, Australia

<sup>5</sup>Melbourne Neuropsychiatry Centre, University of Melbourne & NorthWestern Mental Health, Parkville, Victoria, Australia

<sup>6</sup>Unit of Rare Neurodegenerative and Neurometabolic Diseases, Fondazione IRCCS Istituto Neurologico "Carlo Besta", Milan, Italy

<sup>7</sup>Department of Pediatrics and Adolescence Medicine, First Faculty of Medicine, General University Hospital Prague, Charles University, Prague, Czech Republic

<sup>8</sup>Department of Child Neurology, Comenius University Children's Hospital, Bratislava, Slovak Republic

<sup>9</sup>Department of Neurology, Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat, Spain

<sup>10</sup>Pediatric Neurology Division, Children's Medical Center, Pediatric Center of Excellence, Myelin Disorders Clinic, Tehran University of Medical Sciences, Tehran, Iran

<sup>11</sup>SphinCS GmbH, Clinical Science for LSD, Hochheim, Germany

<sup>12</sup>Actelion, a Janssen company of Johnson & Johnsons, Bern, Switzerland

<sup>13</sup>German Center for Vertigo and Balance Disorders and Department of Neurology, University Hospital Munich, Campus Grosshadern, Ludwig-Maximilians-University Munich, Munich, Germany

## Correspondence

Tatiana Bremova-Ertl, Department of Neurology, University Hospital Bern (Inselspital) and University of Bern, Bern, Switzerland.  
Email: tatiana.bremova-ertl@insel.ch; bremova@gmail.com

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## Abstract

**Objective:** To characterize ocular motor function in patients with Niemann-Pick disease type C (NPC).

**Methods:** In a multicontinental, cross-sectional study we characterized ocular-motor function in 72 patients from 12 countries by video-oculography. Interlinking with disease severity, we also searched for ocular motor biomarkers. Our study protocol comprised reflexive and self-paced saccades, smooth pursuit, and gaze-holding in horizontal and vertical planes. Data were compared with those of 158 healthy controls (HC).

**Results:** Some 98.2% of patients generated vertical saccades below the 95% CI of the controls' peak velocity. Only 46.9% of patients had smooth pursuit gain lower than that of 95% CI of HC. The involvement in both downward and upward directions was similar (51°/s (68.9, [32.7–69.3]) downward versus 78.8°/s (65.9, [60.8–96.8]) upward). Horizontal saccadic peak velocity and latency, vertical saccadic duration and amplitude, and horizontal position smooth pursuit correlated best to disease severity. Compensating strategies

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such as blinks to elicit saccades, and head and upper body movements to overcome the gaze palsy, were observed. Vertical reflexive saccades were more impaired and slower than self-paced ones. Gaze-holding was normal. Ocular-motor performance depended on the age of onset and disease duration.

**Conclusions:** This is the largest cohort of NPC patients investigated for ocular-motor function. Vertical supranuclear saccade palsy is the hallmark of NPC. Vertical upward and downward saccades are equally impaired. Horizontal saccadic peak velocity and latency, vertical saccadic duration and amplitude, and horizontal position smooth pursuit can be used as surrogate parameters for clinical trials. Compensating strategies can contribute to establishing a diagnosis.

#### KEYWORDS

biomarkers, Niemann-Pick type C, ocular motor function, saccades, supranuclear vertical gaze palsy, supranuclear vertical saccade palsy, video-oculography

## INTRODUCTION

Eye movement abnormalities are sensitive markers in neurodegenerative disorders. One such rare autosomal recessive inborn error of metabolism is Niemann-Pick disease type C (NPC), caused by a mutation in the NPC1 (95%) or NPC2 genes. Defects result in a cellular accumulation of endocytosed unesterified cholesterol and glyco- and sphingolipids in the late endosome/lysosome [1]. NPC is characterized by heterogenous manifestations that comprise visceral, neurological, and psychiatric signs. Symptoms are diverse, arise at different ages, and progress at various rates [2].

Previously, vertical supranuclear gaze palsy (VSGP), but not vertical supranuclear saccade palsy (VSSP), was highlighted as the hallmark symptom of NPC at all disease stages [3,4], although the predominant vertical saccadic impairment was described in several case studies [5–7]. VSSP is caused by the impairment of burst neurons (or their downstream/upstream connections) in the rostral interstitial nucleus of the medial longitudinal fascicle (riMLF) in the mesencephalon [8]. The burst neurons in the riMLF play a key role in the generation of vertical saccades and vertical quick phases of nystagmus. VSGP denotes a combined dysfunction of both saccades and smooth pursuit caused by an impairment of the interstitial nucleus of Cajal (InC) in the rostral midbrain and its connections through the posterior commissure [9,10].

For the approval of miglustat in treatment of NPC, horizontal saccadic dysfunction was used as a surrogate marker [11]. Until now, only single cases or small cohorts of NPC patients were thoroughly examined for saccadic function [3–6,12]. Little is known about the non-saccadic ocular motor systems, such as smooth pursuit and gaze holding, and how any deficits relate to disease severity. In contrast to saccadic ocular-motor deficits, vestibulo-ocular reflex of the horizontal semicircular canal and the otoliths are intact in patients with NPC [13]. Degeneration in NPC is not restricted to the ocular-motor system, but also affects retinal axonal function [14].

This cross-sectional prospective study in a large international cohort of patients with NPC had the following objectives. First,

characterize ocular motor function. Reflexive and self-paced saccades, smooth pursuit, and gaze holding, both vertically and horizontally, were examined.

Second, clarify whether VSSP or VSGP is the hallmark of NPC disease, as not all clinicians routinely test for saccadic function in ocular-motor examinations and conventional clinical assessment of gaze range uses smooth pursuit. Third, correlate ocular-motor dysfunction with the severity of disease to identify surrogate biomarkers.

## PATIENTS AND METHODS

### Study population

Of 82 patients screened, 72 patients with genetically and/or biochemically confirmed NPC disease (30 female, 42 male, age  $\pm$  SD  $28.7 \pm 14.2$  years, mean disease duration  $13.2 \pm 9.0$  years, mean age at the establishment of the diagnosis  $22.2 \pm 15.3$  years) were included. Patients who clearly were the most severely impaired, practically doing no activity by themselves (patients bound to wheelchair, not speaking, with feeding tube, and severe dementia), were not screened. These patients were originally (not referring to study sites) from Germany (32.46%), Slovakia (1.44%/2 patients), Czech Republic (15.3%), Greece (2.8%), Italy (5.6%), Bulgaria (0.72%/1 patient), Saudi Arabia (0.72%/1 patient), Spain (18%), Sweden (0.72%/1 patient), Turkey (1.44%/2 siblings), Iran (12.5%), and Australia (8.3%). The characteristics of all patients are listed in Table S1. The mean age at first symptoms of the disease was 14.7 years (SD  $\pm$  95% CI 11.1, [12.1–17.3]). The first neurological symptoms appeared at the age of 16.6 years (12.9, [13.5–19.7]) and the first psychiatric symptoms appeared at the age of 19.0 years (10.4, [14.9–23.1]).

Some 68 of the 72 included patients had a mutations in NPC1, and one patient had an additional mutation in NPC2 gene p.E20\*. The highly frequent mutations p.I1061T and p.P1007A were

present in one patient (p.I1061T). Filipin staining was performed in 64.6% of all patients: it was variant in 20% and classical in 30.8%. In addition, to establish the diagnosis and disease development, elevated levels of lysosphingolipids were detected in 9 (9.7%), oxysterols in 17 (23.6%) and, most commonly, chitotriosidase in 25 patients (34.7%). Most patients were on medication with miglustat (62 patients, 86.1%). The mean duration ( $\pm$ SD, 95% CI) of miglustat treatment was 3.9 years (3.1, [2.9–5.0]). Twenty-eight patients (68.3%) were on miglustat for longer than 1 year and 13 (31.7%) for less than 1 year.

Regarding the NPC form, 2 patients (3.2%) were early-infantile, 5 (7.8%) late-infantile, 26 (40.6%) juvenile, and 39 (48.4%) adult (>15 years of age). Twenty-four patients (34.3%) had a first degree relative with NPC disease. Six patients had cousins with NPC (8.6%) and 3 patients (4.3%) had higher-degree relatives with NPC.

The neurological symptoms observed (by the supervising clinician) were: VSGP in 41 (56.9%) and VSSP in 48 patients (59.3%), gelastic cataplexy in 9 (12.7%), clumsiness or frequent falls in 52 (72.2%), dysidiadochokinesis in 49 (70.0%), dysmetria in 50 (71.4%), intentional tremor in 29 (42.0%), and dysarthria and/or dysphagia in 63 (88.7%). Dyskinesia was observed in 26 cases (37.7%), dystonia in 48 cases (67.6%), and acquired spasticity in 15 patients (21.1%). Delayed developmental milestones were observed in 13 cases (18.3%). Fifteen patients (20.8%) demonstrated partial or generalized seizures, 9 patients (12.5%) showed myoclonus. Some 62 patients (86.1%) demonstrated cognitive decline (children) or dementia (adults). Psychotic symptoms (hallucinations, delusions and/or thought disorder) were present in 16 patients (19.5%), 9 patients (12.5%) showed treatment-resistant psychiatric symptoms, whereas disruptive or aggressive behavior was observed in 24 patients (29.3%). Other psychiatric disorders were seen in 14 patients (19.7%). Patients' medication included a number of central nervous system (CNS)-active agents: antiepileptics (9 patients, 12.5%), antidepressants (11, 15.3%), nootropics (7, 9.7%), antipsychotics (10, 13.9%), anti-Parkinsonian medication (including anticholinergics), and benzodiazepines (4, 5.6%). Three patients had hearing devices due to hearing loss. Acetyl-DL-leucine was administered in 21 patients (31.7%) for cerebellar ataxia [15]. One patient was treated with cyclodextrin, while ursodeoxycholic acid (UDCA) was administered to a further patient (early-infantile case with profound visceral manifestation).

For a healthy control as a reference, 158 subjects from Germany and Slovakia with no presence of neurological, ophthalmological, or vestibular disease (55 F, age  $\pm$  SD 41.7  $\pm$  24.3 years) were also included to build up 95% confidence limits using the same testing paradigms.

The study was performed in accordance with the Helsinki II Declaration and approved by the ethics committee of the Ludwig Maximilian University Medical Faculty (addendum to project no. 379–12). The principal investigator (T.B.E.) initiated the study during her work in Munich, before she moved to Bern (see the Affiliation section). All participants or their legal guardians gave their informed consent prior to inclusion in the study.

## Eye movement recordings and data analysis

For eye movement recordings, paradigms and all definitions of ocular-motor parameters, see Appendix S1.

## Clinical evaluation

To assess disease severity, the modified Disability Rating Scale (mDRS) was applied [16]. Cerebellar function was evaluated by the Scale for the Assessment and Rating of Ataxia (SARA) [17] and the Spinocerebellar Ataxia Functional Index (SCAFI) [18]. To evaluate cognitive function, the Montreal Cognitive Assessment (MoCA) was used [19].

## Statistical analysis

Statistical analysis and figure design were performed using SAS<sup>®</sup> Software version 9.3. Graph design was performed by GraphPad<sup>®</sup> Software version 5.0.3 (La Jolla, CA). Numerical variables in demographic and baseline characteristics were summarized by displaying the following: *n* (actual sample size), mean, standard deviation, standard error, median, quartiles, 95% confidence intervals, and maximum and minimum. We reported the frequency and percentages (based on the non-missing sample size) of observed levels for all categorical measures. Parametric *t*-test was used to determine differences in the mean value between NPC patients and HC. Analysis of variance (ANOVA) was used to determine whether a mean value was statistically different among NPC subpopulations (i.e., early-infantile, late-infantile, juvenile, and adults).

To assess which eye movement parameters could predict the severity of patients with NPC (i.e., correlation between video-oculographic impairment and disability scale), we used them as covariates in the univariable linear regression analysis. To study whether clinical parameters played a significant role in such correlations, we ran multivariable regression analysis with a stepwise and forward selection method. In linear regression models, *R*<sup>2</sup> of the linear regression line including controls are indicated.

We performed an observed case analysis, with all summary and percentages calculated relative to the number of patients with available ocular-motor data. In the resulting statistics, the denominators for analysis were the numbers of patients with the corresponding data. Patients who were not physically capable of performing the particular score tasks, or did not perform the test for other reasons, were excluded from the analysis.

## RESULTS

### Clinical state

The mean mDRS score was 10.3 points (SD, 95% CIs of the mean 5.0, [9.4, 11.5]) (*n* = 69), the mean SARA score was 13.3 points (9.4, [10.9, 15.6]) (*n* = 63). The mean MoCA (*n* = 31) was 16.7 points (7.4, [14–19.4]).

## Saccades

For a normal subject as a reference, see Figure 1A. As illustrated in Figure 1B–E, we observed different patterns of saccadic impairment in patients with NPC. These patterns comprised hypometric (B), staircase-pattern (C), ‘normal slow’ (D), and pursuit-like pattern (E) (extremely slow, but still movement generated) saccades. As a compensating strategy, patients generated blinks to a very significant extent (F). Saccades with blinks were excluded from the analysis. Clinically observed head movements were avoided during the examination by chinrest and active help of the examiner/caregiver (Appendix S1). The trajectory of saccades can be seen in phase-plane plots of velocity versus amplitude (Figure 2). For a reference, see normal subjects (2A and 2B). We observed velocity fluctuation, visible as “wiggles”, both in reflexive and self-paced vertical saccades (2C and 2E), but not in the horizontal ones (2D and 2F).

### Reflexive vertical saccades

Sixty-one patients were able to perform vertical saccades (73.2%) of 10° amplitude and 58 patients generated saccades to 20° ( $\pm 10^\circ$  up and down from center of 0°). Mean peak velocity of saccades in response to stimulus of 20° was 63.5°/s (59.5, [47.9–79.2]) in NPC patients and 403.1°/s (69.0, [392.0–414.2°/s]) in HC ( $p < 0.001$ ). Downward saccades yielded the peak velocity of 51°/s (68.9,

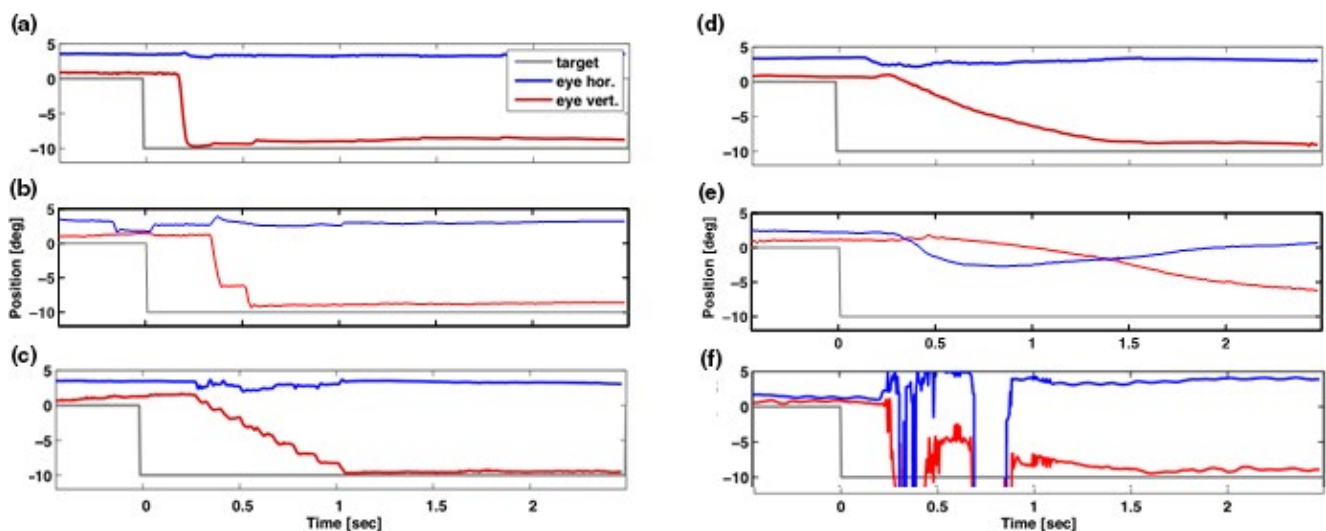
[32.7–69.3]), whilst upward saccades measured 78.8°/s (65.9, [60.8–96.8]) (for both:  $p < 0.001$ ). For saccadic gain (saccadic amplitude/stimulus amplitude), see section on “Vertical Saccades and Vertical Smooth Pursuit” below. The overall characteristics of vertical saccades differed when compared with healthy controls at  $p < 0.001$  levels, except for the latency ( $p < 0.05$ ).

### Reflexive horizontal saccades

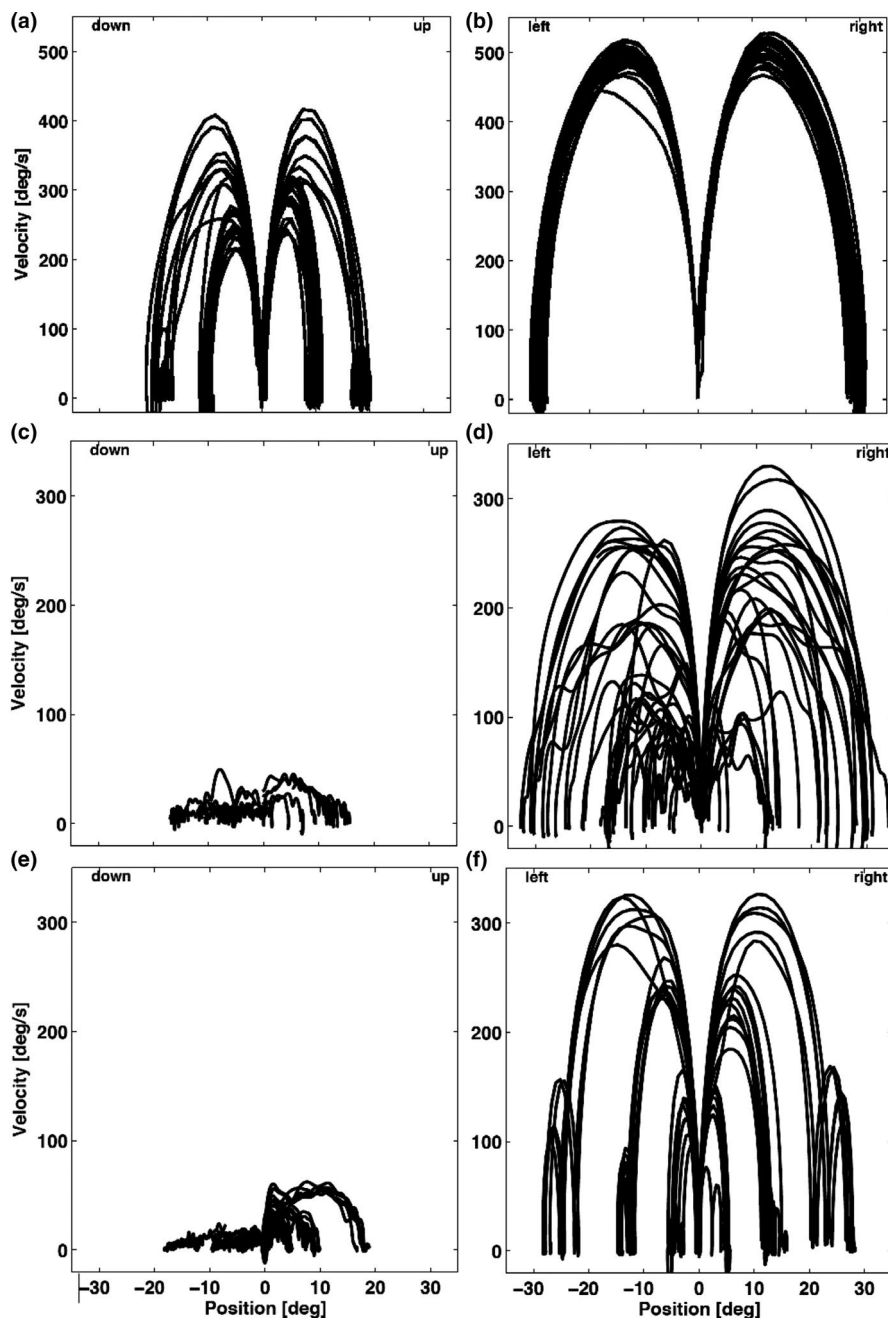
Peak velocity of horizontal saccades in response to 30° was 311.7°/s (103.4, [284.6–338.9]) in patients with NPC and 481.7°/s (78.1, [468.8–494.5]) in HC ( $p < 0.001$ ) (for both 15° and 30° saccades). All other saccadic characteristics (amplitude, duration, and maximal duration) except latency showed a significant difference.

### Self-paced vertical saccades

Peak velocity of upward self-paced saccades to 20° was 158.8°/s (106.2, [39.9, 322]) in NPC patients and 352.7°/s (85.4, [251–459]) in HC ( $p < 0.001$ ). Peak velocity of downward self-paced saccades reached 162.3°/s (146.7, [292.7–106.2]) in NPC and 352.8°/s (86.4, [458.5–236]) in the control group ( $p < 0.001$ ). In contrast to reflexive saccades, NPC patients showed a prolonged intersaccadic interval of self-paced saccades (as an equivalent of latency in reflexive



**FIGURE 1** Overview of saccadic phenotypes in a healthy control and in NPC patients: A continuum. Phenotypes of vertical downward saccades in NPC disease are depicted. Red indicates the vertical eye movement and blue the horizontal eye movement. Grey depicts the stimulus. Figure A shows a vertical downward saccade of a normal subject. Note the high peak velocity (steep line) trajectory without fluctuations, latency  $< 250$  ms, amplitude and precision without hypo- or hypermetria, and trajectory without curving. A common finding in NPC is saccadic hypometria (B)  $> 20\%$  of the whole saccade. If hypometria is very profound, so-called staircase saccades, seen also often in patients with atypical Parkinson syndromes, are generated (C). Also notice a prolonged latency (time from the stimulus onset to the initiation of the downward movement). Reduced velocity of vertical saccades is depicted in (D), with a downward saccadic palsy in E. Note that that there is still extremely slow, pursuit-like movement, and the actual saccade performance takes more than two seconds. One of the most remarkable strategies seen in NPC is *blinking* (F). This strategy is used to initiate saccades that are already impaired. In contrast, patients with parkinsonian disorders do not blink to compensate for saccadic palsy due to lid apraxia or blepharospasmus. Note the continuum of saccadic impairment with different saccadic phenotypes. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



**FIGURE 2** Phase plane plot of vertical saccades in a normal subject and in a patient with Niemann-Pick type C. Phase-plane plots depicting the saccadic amplitude vs velocity. The origin of each saccade has been moved to zero and the velocity at each position along the saccade trajectory is shown. Since larger amplitude saccades typically generate faster velocities, this generates a family of curves in which the saccade amplitude can be seen at the endpoint position. Note also the velocity fluctuation of saccades (wiggles), as shown in the phase-plane plot. The velocity variation ("wiggles") is higher in vertical reflexive (C) than in vertical self-paced (E) saccades. This illustrates the difference between the reflexive and self-paced saccades.

saccades). For upward saccades, this was 2.5 s (1.4, [1.2–2.6]) in NPC and 1.1 s (0.4, [0.8–1.6]) in HC. Regarding the downward saccades, it was 3.5 s in NPC (5.0, [1.2–2.6]) and 1.03 s (0.6, [0.8–1.9]) in HC ( $p < 0.001$ ). Patients generated a total mean number of 10.6 (19, [2–27]) upward saccades and 8.1 (9.0, [1.0–29.0]) downward saccades in 30 s, whereas controls generated 30.6 (8.3, [19–41]) upwards and 25.8 (8.7, [16–38]) downwards, respectively (for all  $p < 0.001$ ).

### Self-paced horizontal saccades

Peak velocity of rightward self-paced saccades yielded 244.6°/s (138.6, [179.7–309.5]) in NPC and 449.8°/s (97.2, [432.7–466.8])

in HC. To the left, peak velocity reached 240°/s (152.7, [168.5–311.5]) in NPC and 429.8°/s (112.3, [410.1–449.1]) in HC. As expected, the intersaccadic interval was significantly prolonged in NPC. To the right, it yielded 2.1 s (1.4, [1.5–2.8]) in NPC and 1.4 s (0.7, [1.2–1.5]) in HC. To the left, the intersaccadic interval was 1.8 s (1.1, [1.3–2.4]) and 1.3 s (0.5, [1.2–1.4]) (for both  $p < 0.001$ ). The absolute number of elicited saccades to the right generated in 30 s was 15.7 (8.6, [11.6–19.7]) in NPC and 25.7 (8.7, [24.1–27.2]) in HC ( $p < 0.001$ ). To the left, the absolute number was 14.6 (8.6, [10.6–18.6]) in NPC and 26.5 (67.3, [91.1–114.8]) in HC ( $p < 0.001$ ). In all the other saccadic parameters, there were significant differences when compared to HC ( $p < 0.001$ , for amplitude  $p < 0.002$ ).



## Reflexive vs. self-paced saccades

We ascertained whether there was a significant difference between the two types of examined saccades in NPC. For vertical reflexive saccades, peak velocity was 63.5°/s (59.5, [47.9–79.2]). In contrast, peak velocity of vertical self-paced saccades was 160.6°/s (126.5, [166–214]) ( $p < 0.001$ ) (for an example, see Figure 2C reflexive vs. 2E self-paced). Interestingly, this was not true for horizontal saccades. For reflexive saccades, mean peak velocity was 311.7°/s (103.4, [284.6–338.9]). In the self-paced task, peak velocity reached 242.3°/s (145.7, [174.1–310.5]).

## Smooth pursuit

Vertical velocity smooth pursuit gain in response to 0.2 Hz stimulus frequency was 0.544 (0.269, [0.467–0.621]) in NPC and 0.572 (0.2, [0.54–0.604]) in HC ( $p = 0.435$ ). However, vertical position smooth pursuit gain was 0.649 (0.33, [0.554–0.744]) in NPC and 0.935 (0.149, [0.91–0.959]) in HC ( $p < 0.001$ ). Horizontal velocity smooth pursuit gain in response to 0.2 Hz was 0.619 (0.207, [0.546–0.693]) in NPC and 0.812 (0.192, [0.781, 0.843]) in HC ( $p < 0.001$ ). Horizontal position smooth pursuit gain to 0.2 Hz stimulus frequency was 0.979 (0.213, [0.897, 1.060]) in NPC.

## Comparison of vertical saccades and vertical smooth pursuit

98.2% of patients generated vertical saccades (both up and down) that were below the 95% confidence intervals of the controls' peak velocity. This was not the case for smooth pursuit generated in response to 0.2 Hz stimulation, where only 46.9% of patients had smooth pursuit gain lower than that of 95% of HC. Gain (eye movement amplitude vs stimulus amplitude) of vertical saccades in NPC patients was 0.525 (0.282, [0.451–0.599]) and lower than smooth pursuit gain 0.649 (0.330, [0.554–0.744]) ( $p = 0.038$ ), while this was not true for HC (vertical saccadic gain: 0.953, (0.112, [0.935–0.971]) vs smooth pursuit gain 0.935, (0.149, [0.910–0.959]);  $p = 0.234$ ).

For a juvenile-onset patient with NPC, suffering VSSP without VSGP, see Video S1. Signed informed consent was obtained from the patient.

## Gaze-Holding

No significant gaze-holding deficit was observed.

## Oculomotor characteristics based on age of onset

Due to the low number of early-infantile and late-infantile cases (12  $\leq$  age, in total 12, results of 7 of them were analyzable), thus

reducing the probability of ANOVA statistically significant results, these groups were excluded from this analysis.

The characteristics of 10° saccades did not differentiate based on age of onset. 20° saccades differed in terms of amplitude of both upward and downward saccades, respectively ( $p = 0.035$ ;  $p = 0.022$ ). There was a trend for significance of velocity of upward saccades ( $p = 0.057$ ), and downward amplitude ( $p = 0.051$ ). 20° mean vertical saccadic velocity ( $p = 0.022$ ) and amplitude ( $p = 0.016$ ) were significantly different based on the studied groups. In response to both 15° and 30° stimuli, leftward and rightward saccades did not vary between the studied groups.

Horizontal but not vertical smooth pursuit was lower in juvenile-onset, than in adult-onset cases ( $p < 0.01$ ).

## Univariate linear regression and further analysis

Ocular-motor parameters that showed significant relationships with disease-specific and ataxia scales and  $R^2$  values with the tendency to significance are summarized in Table S2. All relationships (video-oculographic parameters vs neurological scales) were plotted and outcomes with significant  $p$  values and higher  $R^2$  were evaluated for data dispersion and fit of the regression line.

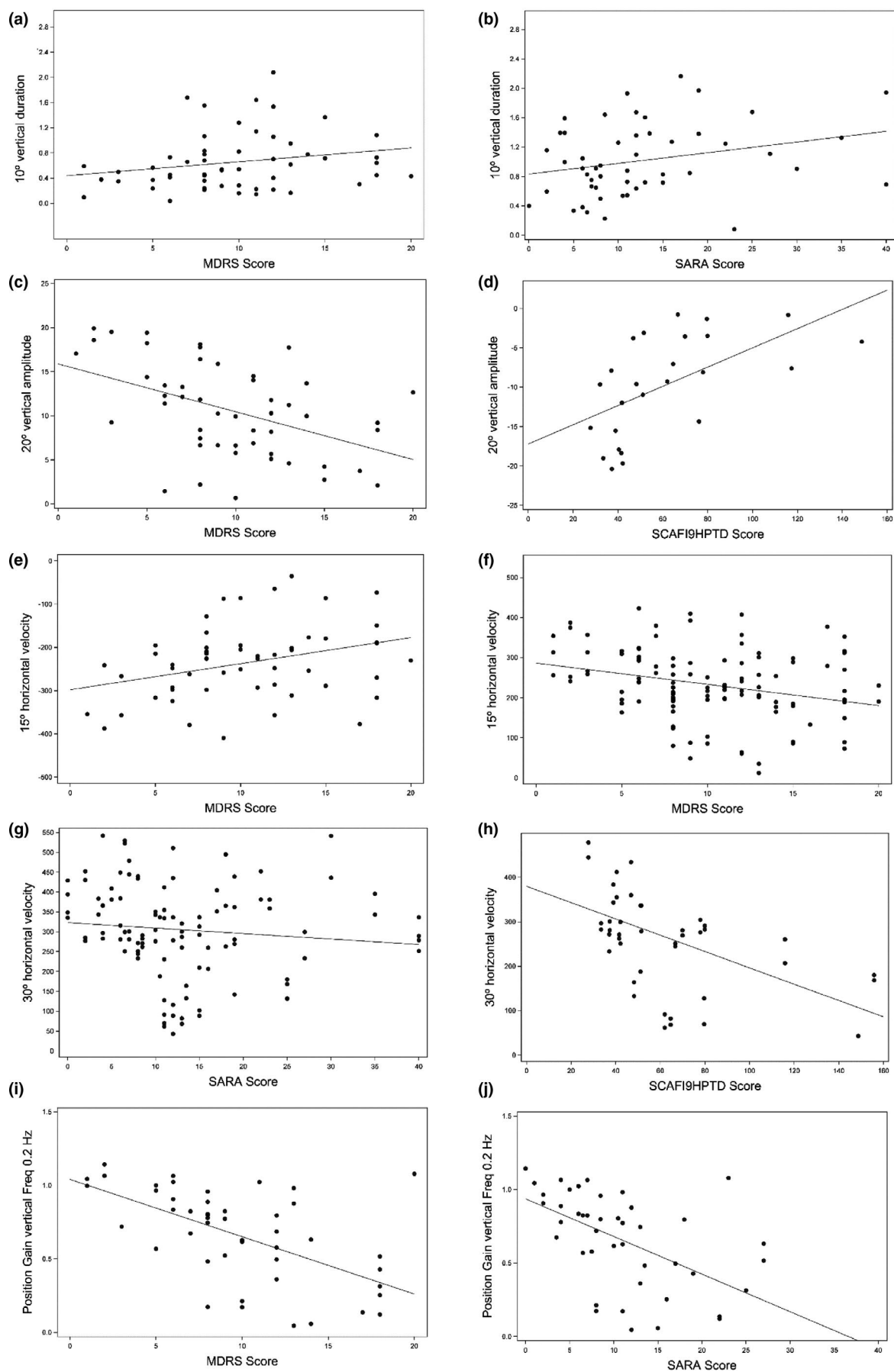
Univariate linear regression analysis showed that duration of 10° saccades is strongly related to disease state ( $R^2 = 0.68$ ) (Figure 3A,B), particularly 10° downward saccades. 20° vertical down amplitude tracked with mDRS ( $R^2 = 0.61$ ) (Figure 3C). 20° vertical saccadic amplitude was a negative predictor to time of SCAFI 9-Hole-Peg-Test of the Dominant Hand (9HPTD) ( $R^2 = 0.58$ ) (Figure 3D), but also mDRS ( $R^2 = 0.58$ ). Peak velocity of vertical saccades was not useful in predicting disease severity (for up/down and 10°/20°,  $R^2 < 0.2$ ).

Peak velocity of 15° left horizontal saccades was significantly related to mDRS scale ( $R^2 = 0.53$ ) (Figure 3E), when combined with 15° horizontal left-right peak velocity ( $R^2 = 0.46$ ) (Figure 3F). Horizontal saccadic combined (left-right) PV and SARA score yielded  $R^2$  of 0.38 (30° saccades) and  $R^2$  of 0.37 (15° saccades) (Figure 3G). SCAFI domain 9HPTD and peak velocity of 15° left-right saccades yielded  $R^2$  of 0.35 (Figure 3H). Latency of 15° rightward saccades tracked with SCAFI 9HPTD Score ( $R^2 = 0.56$ ).

Position smooth pursuit gain to 0.2 Hz stimulation was related to both mDRS and SARA scores to a modest extent ( $R^2 = 0.32$  vs  $R^2 = 0.31$ ) (Figure 3I,J). Gaze-holding did not reflect disease severity.

Miglustat treatment was used as a covariate in the multivariate regression analysis; however, the assessment of its role was hampered by the patient-to-patient variability and restricted sample sizes.

In patients treated  $>1$  year, peak velocity of 10° upward saccades ( $p = 0.046$ ) was significantly lower, probably due to NPC disease duration, *de facto* suffering an advanced disease. Regarding horizontal saccades, we found a lower peak velocity to 15° stimulus (right  $p = 0.048$ ; left  $p = 0.031$ ) and to 30° stimulus ( $p = 0.004$ ).



**FIGURE 3** Saccadic and smooth pursuit parameters and their relationships to neurological scores.

## DISCUSSION

Ocular-motor function in NPC disease has not previously been analyzed in a large cohort of patients due to its rarity and underdiagnosis. We systematically examined reflexive and self-paced vertical and horizontal saccades, smooth pursuit and gaze-holding as functional outputs of the brainstem, cerebellum and cortical centers. The major findings of this study are as follows:

*First*, quantitative measurements of eye movements showed that VSSP, not VSGP, is the cardinal symptom of NPC disease. *Second*, contrary to what is commonly reported, downward *and* upward saccades are impaired to a similar extent. *Third*, reflexive vertical saccades were more impaired and slower than self-paced ones. In contrast, the velocity of horizontal self-paced saccades was lower than their reflexive counterparts. *Fourth*, horizontal saccadic peak velocity and latency, amplitude and duration of vertical saccades, and gain of positional smooth pursuit were the most relevant variables reflecting clinical state. *Fifth*, ocular-motor status was dependent on age of onset and disease duration. *Sixth*, we observed patient-specific saccadic phenotypes, but due to the compound heterozygosity, genotype-ocular-motor phenotype correlation was not possible. Of note, we noticed patients apply blinking, and head and upper body movements to initiate and accelerate the saccades and to overcome gaze difficulty [20,21]. Clinicians should actively search for these clues.

### Vertical Supranuclear Saccadic vs. Gaze Palsy

Prior to this analysis, VSGP but not VSSP has been highlighted as a cardinal symptom of NPC; this however fails to acknowledge the specific ocular-motor impairment characteristic of the disease. The terminology has not been used clearly, potentially contributing to the lack of descriptive accuracy of the saccadic eye movements defect in patients. In clinical practice, the ocular-motor examination is often restricted to following the slowly moving target when looking for gaze limitations, thus examining smooth pursuit, whereby slow vertical saccades may be overlooked. Thus, a thorough ocular-motor examination of both saccades *and* smooth pursuit movements will ensure that VSSP is not missed. Whilst VSGP has been shown to be present in 70% of patients from an international patient registry [22], we demonstrate here that VSSP is present in 98.2% of patients, but VSGP in only 46.9% (Video S1). VSGP is present in advanced stages of ocular-motor impairment, when the disease is already diagnosed.

Vertical saccades are often accompanied by the “around the house” sign (curved saccade trajectory when plotted in X-Y space) (Video S1), typical of saccadic palsy in one plane [23], also seen in other lysosomal storage diseases (LSDs), like Gaucher disease type 3 [24]. Clinicians should be trained to recognize and differentiate impairments of the saccadic system from impairments of smooth pursuit, and understand the possibility of combined deficits. To emphasize the important distinction between VSSP and VSGP, we suggest revising the current terminology with regards to saccadic

vs. gaze palsy also in other LSDs and neurodegenerative diseases like progressive supranuclear palsy (PSP) [25,26]. Saccadic palsy is the initial symptom due to the selective vulnerability of the saccadic neurons, probably due to their high metabolic properties, but the underlying pathophysiology is not known.

### Vertical saccadic patterns

We observed different saccadic patterns that were patient-specific, such as slow saccades, and extremely slow, “pursuit-like” saccades, where the saccade resembled a pursuit movement. Saccade-generating burst neurons are inhibited by omnipause neurons, except during saccadic performance and blinks [27,28]. Due to transient velocity fluctuations during the saccade, suggestive of omnipause neuron dysfunction, we argue the movement was generated by burst neurons in riMLF, rather than the pursuit system. Patients generated hypometric saccades with either one large corrective saccade (>20% of amplitude of first saccade) or so-called staircase pattern saccades. The number of saccadic steps varied from 2 to 10. Hypometric saccades are common for atypical Parkinson syndromes, such as PSP, the main differential diagnosis for the adult form of NPC, supporting the association between NPC and other neurodegenerative disorders [25,26,28].

### VSSP/VSGP compensating strategies

Although not the main objective of this study, we demonstrate that observable compensation strategies patients utilize to overcome ocular-motor deficits can assist in establishing the diagnosis of NPC. To elicit and speed-up the saccades, patients blink to silence omnipause neurons that inhibit burst excitatory neurons. To overcome palsy of gaze, patients produce high-amplitude head movements or even upper body movements. This can often be seen in children [20,21]. We strongly encourage clinicians to look for these movement clues that compensate for subclinical ocular-motor impairment, which should prompt thorough ocular-motor and neurological examinations.

### Reflexive vs. Self-paced saccades

Peak velocity of vertical, but not horizontal, self-paced and reflexive saccades in response to the same amplitude significantly differed. This is surprising, since burst neurons for all saccades have a common supranuclear center, namely riMLF for vertical and pontine paramedian reticular formation (PPRF) for horizontal saccades [10]. The firing rate of the excitatory burst neurons is linearly aligned with the saccadic velocity [29]. The reason self-paced saccades, a marker for degenerative and traumatic brain disorder [30–32], reach higher peak velocity is unclear, considering impaired cognition and processing speed in NPC.



There may be different involvement of supra-riMLF pathways, namely frontal eye field for self-paced and parietal eye field for reflexive saccades [3,33]. The characteristics of both self-paced and reflexive saccades were pathological when compared to the healthy population. We speculate that the rapidly changing visual stimulus for highly disturbed vertical reflexive saccades might represent a relevant stressor that compromises the performance. Horizontal saccades also showed a remarkable decrease of peak velocity and prolonged duration, demonstrating the functional impairment of the PPRF in the pons, but they are affected to a lesser extent.

As expected, latency in vertical reflexive saccades was normal, but the intersaccadic interval was greatly prolonged. This supports the progressive frontal impairment in NPC.

### Upward vs. Downward saccades

In contrast to the commonly perceived discrepancy between downward and upward saccades due to the mono-(ipsi-) and bilateral supranuclear innervation of oculomotor nuclei by the riMLF, both downward and upward saccades showed similar characteristics, including peak velocities. This might be because the bilateral upward innervation is counteracted by gravity. In neurodegeneration, the same degree of functional impairment results in both directions. Less frequent upward eye movements might lead to a worse upward motor function, when compared with common downward eye movements, due to a training-effect.

### Use as biomarkers

Even though vertical saccades show similar peak velocities in both directions, the duration of downward saccades correlates with the state of disease more than the duration of upward ones. Our results suggest that position, not velocity smooth pursuit profile, can be established as a surrogate parameter. Interestingly, this is more the case for vertical, not horizontal smooth pursuit. The characteristics of self-paced saccades might be a useful biomarker, especially total count and peak velocity. Further ocular-motor studies using this task are needed. In terms of important covariants, disease duration affected the ocular-motor systems to a considerable extent, as expected. Additionally, ocular-motor function in NPC1 heterozygotes (one mutation carriers) is similar to patients with manifest disease (with two underlying mutations), replicating the neurodegenerative pattern [34]. Thus, the identified ocular-motor parameters can be used in NPC1 heterozygotes to screen for neurological impairments. Once created, the parameters may be used to evaluate disease progression treatment effects.

### Limitations

The primary limitation of this study was the disease complexity and level of the patients' disability, with incomplete data in some

patients. We admit that not including the most severely impaired patients might represent a selection bias. However, these patients have often already a total gaze palsy, including horizontal eye movements so that the ceiling effect of the video-oculographic testing would be probably reached. Many patients utilized dopaminergic, anti-epileptic and anti-psychotic medication. Medication effects on ocular-motor function are expected, but due to a number of diverse medications, we were not able to study their functional relevance.

Dividing the patient population into groups led to smaller sample sizes, thus reducing the power of the study. The cross-sectional and uncontrolled design of the study is a limitation in this progressive neurodegenerative disease.

## CONCLUSIONS

The cardinal ocular-motor sign of NPC disease is VSSP. All patients with VSGP demonstrate a saccadic impairment, but not all patients with VSSP show gaze palsy.

Parameters that could serve as surrogate endpoints are horizontal saccadic characteristics, with position, vertical smooth pursuit gain and vertical saccadic duration, and amplitude as secondary endpoints. VSSP can help clinicians diagnose NPC in its initial stages. Early diagnosis is essential so that disease-modifying treatment, which slows neurologic progression and prolongs life-expectancy, can be initiated before the window of therapeutic opportunity is lost.

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### DISCLOSURE

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## AUTHOR CONTRIBUTIONS

**Tatiana Bremova-Ertl:** Conceptualization (lead); Investigation (lead); Methodology (lead); Project administration (lead); Software (supporting); Visualization (equal); Writing-original draft (lead); Writing-review & editing (lead). **Larry A. Abel:** Data curation (lead); Formal analysis (equal); Methodology (supporting); Resources (equal); Validation (equal); Writing-review & editing (supporting). **Mark Walterfang:** Investigation (equal); Project administration (equal); Writing-review & editing (supporting). **Ettore Salsano:** Investigation (equal); Project administration (equal). **Anna Ardissonne:** Investigation (equal); Project administration (equal). **Jordi Gascon:** Conceptualisation (equal); Project administration (equal). **Vera Malinova:** Investigation (equal); Project administration (equal). **Miriam Kolnikova:** Investigation (equal); Project administration (equal). **Ali Reza Tavasoli:** Investigation (equal); Project administration (equal); Writing-review & editing (supporting). **Mahmoud Reza Ashrafi:** Investigation (equal); Project administration (equal). **Yasmina Amraoui:** Investigation (equal); Project administration (equal); Writing-review & editing (supporting). **Eugen Karl Mengel:** Investigation (equal); Project administration (equal). **Stefan Kolb:** Conceptualization (supporting); Funding acquisition (equal); Methodology (supporting); Resources (supporting). **Andreas Brecht:** Conceptualization (supporting); Funding acquisition (equal); Methodology (supporting); Resources (equal). **Stanislavs Bardins:** Conceptualization (supporting); Data curation (lead); Formal analysis (lead); Methodology (supporting); Visualization (equal). **Michael Strupp:** Conceptualization (equal); Funding acquisition (lead); Methodology (supporting); Resources (lead); Writing-review & editing (supporting).

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## ORCID

Tatiana Bremova-Ertl  <https://orcid.org/0000-0002-7397-045X>

Larry Abel  <https://orcid.org/0000-0002-2393-0644>

Mark Walterfang  <https://orcid.org/0000-0002-1389-3691>

Ettore Salsano  <https://orcid.org/0000-0001-9167-1878>

Anna Ardissonne  <https://orcid.org/0000-0003-1969-0147>

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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