

SUPPLEMENTARY MATERIAL

**Clonazepam repurposing in *ARID1B* patients through conventional RCT and N-of-1 Trials -
An experimental strategy for orphan disease development**

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Table S1: *ARID1B* variants of included patients

<i>ARID1B</i> cDNA variant	<i>ARID1B</i> protein change	inheritance
NC_000006.11:g.(?_157144644)_(158028969_?)del	p.0	<i>de novo</i>
NM_020732.3:c.1146dup	p.(Gly383Argfs*152)	<i>not tested</i>
NM_020732.3:c.1160_1200del	p.(Ala387Glyfs*134)	<i>de novo</i>
NM_020732.3:c.1202delG	p.(Gly401Alafs*29)	<i>de novo</i>
NM_020732.3:c.1320C>G	p.(Tyr440*)	<i>de novo</i>
NM_020732.3:c.1579C>T	p.(Gln527*)	<i>de novo</i>
NM_020732.3:c.2318C>G	p.(Ser773*)	<i>de novo</i>
NM_020732.3:c.2371+5G>A	r.(spl?)	<i>not tested</i>
NM_020732.3:c.2519dup	p.(Tyr840*)	<i>de novo</i>
NM_020732.3:c.2917dup	p.(Met973Asnfs*16)	<i>not tested</i>
NM_020732.3:c.3478G>T	p.(Glu1160*)	<i>not tested</i>
NM_020732.3:c.4623_4641dup	p.(Asn1548Aspfs*94)	<i>de novo</i>
NM_020732.3:c.4870C>T	p.(Arg1624*)	<i>de novo</i>
NM_020732.3:c.5072del	p.(Leu1691Argfs*75)	<i>de novo</i>
NM_020732.3:c.5404C>T	p.(Arg1802*)	<i>de novo</i>
NM_020732.3:c.5508del	p.(Ser1836Argfs*15)	<i>de novo</i>

Table S2. Schedule of assessment

	SCR		Study period 1 & 2					
			Day 1 & 22					Day 2-21
Time	-60 days ⁴	-1 h	0 h	1h	3h	4.5h	5h	
Assessments								
Informed consent	X							
Demography	X							
Inclusion and exclusion criteria	X							
Medical history	X							
Symptoms	X	X	X	X	X		X	
Arrival in clinic		X						
Explanation of NeuroCart and study procedures	X	X						
Vital signs (Weight, length, HR, BP & temp)		X						
Adverse events			X	X	X		X	X ⁷
Questionnaires ¹		X						
Drug administration (oral)			X					X ⁸
Cognitive test battery ³		X		X	X		X	
Rinse mouth		X				X	X	
Saliva sample PK		X ⁶				X	X	
Discharge							X	
trial@home device explanation to parents							X	
trial@home ²								X
Teacher/caregiver questionnaire								X ⁵

SCR = screening; PK = pharmacokinetic; HR = Heart rate; BP = Blood pressure

1. Questionnaires consist of: ABC questionnaire, and the Clinician's Global Impression (CGI-S at day 1, CGI-I at day 22).
2. Continuous physical activity, heart rate and sleep monitoring, as well as twice-weekly finger tapping, adaptive tracking and animal fluency.
3. NeuroCart tests consist of: animal fluency test, adaptive tracking, body sway, saccadic eye movements, smooth pursuit and tapping frequency.
4. Screening by phone.
5. ABC questionnaire on paper. Interview by phone for CGI-S (> day 1) and CGI-I (> day 21), conducted as soon as possible after the study-day.
6. Only on day 22
7. Evaluation adverse events by phone by study physician at Day 3 and Day 6 and when needed
8. Starting on the evening of Day 22, clonazepam will be tapered by decreasing the daily dose by 0.01 mg/kg/day every three days.

Table S3: Self-reported improvements reported during CGI-I interview

Reported improvements	Clonazepam <i>n</i> =7	Placebo <i>n</i> =2
Expressive speech	4	1
Mental calmness	3	
Emotional outbursts/more susceptible to reason	2	
Concentration	2	1
Taking initiative	1	2
Indicating boundaries	1	
Sleep	5	

Table S4: Example of a SMART-defined goal using Goal Attainment Scaling

Goal attainment level	Score	SMART Goal X
Less than baseline	-3	[name] can concentrate on schoolwork for less than 3 consecutive minutes.
Baseline	-2	[name] can concentrate on schoolwork for about 3 minutes straight.
Goal partially achieved	-1	[name] can concentrate on schoolwork for 3-5 consecutive minutes.
Desired outcome	0	[name] can concentrate on her schoolwork for 5-10 consecutive minutes.
Somewhat better than desired outcome	1	[name] can concentrate on her schoolwork for 10-12 consecutive minutes.
Much better than desired outcome	2	[name] can concentrate on her schoolwork for >12 consecutive minutes.
Weight (1=important; 2=very important; 3=most important)	3	Most important
Probability of goal attainment (1=doubtful; 2=possible; 3=probable)	2	Possible

Table S5: N-of-1: Patient characteristics

	Sex	Age	doses RCT	doses N-of-1
Subject 1	Female	25	2dd3	2dd4
Subject 2	Male	15	2dd6	2dd3
Subject 3	Female	12	2dd6	2dd4

Table S6: Treatment goals (ICF)

ICF level goals		Placebo	Blinded period/C	Clonazepam
	Total number of goals*	Number of (partially) achieved goals**	Number of (partially) achieved goals**	Number of (partially) achieved goals**
Body functions and structures				
Sleep	2	1	2	2
Energy level	1	0	0	1
Concentration	2	0	1	2
Reaction time	1	0	1	0
Mental calmness***	1	NA	NA	NA
Activities and participation				
Communication/talking	2	0	1	1
Emotional fits	1	0	1	1
Social contact	1	1	0	1
Personal factors				
Dealing with anxiety	1	0	1	1

ICF: International Classification of Functioning, Disability and Health; NA=not applicable

* Subgoals were excluded

** average over period

*** not possible to evaluate

Table S7: Primary outcome

Subject	Placebo		Blinded period - clonazepam		Clonazepam		p-value*	
	GAS-T baseline	Average GAS-T	Average GAS-T		Average GAS-T		Placebo vs clonazepam (total study period)	Placebo vs clonazepam (blinded only)
Subject 1	23,5	24,5	27,3		32,2		0.06	0.19
Subject 2	19,4	23,0	31,2		35,9		0.04	0.18
Subject 3	19,4	21,5	51,3		56,4		0.03	<0.01
Group analysis							<0.01	<0.01

* p-value of GAS-T change from baseline

Table S8: Behavioral questionnaires in the N=1 study

Outcomes	Baseline		Placebo		Double-blind clonazepam		Clonazepam				
<u>ABC</u>	<i>n</i> =3										
1: Irritability	6,00	8,72	6,06	9,08	6,33	8,50	0,83	1,04	0,05	a	
2: Lethargy	14,67	9,45	14,50	9,26	16,50	12,50	8,58	7,47	0,11	a	
3: Stereotypy	1,33	1,53	0,83	3,21	0,67	1,15	0,17	0,29	0,07	a	
4: Hyperactivity	11,67	5,69	9,94	5,76	12,00	5,41	7,33	2,84	0,14	a	
5: Inappropriate Speech	2,33	3,21	2,17	2,93	2,00	2,65	0,08	0,14	0,06	a	
<u>CBCL</u>	<i>n</i> =2**										
<i>Subscales</i>											
1: Anxious/depressed	4,00	4,24	1,83	1,18	2,25	1,77	1,50	0,71	0,46	a	
2: Withdrawn/depressed	6,00	2,83	6,00	3,30	6,25	3,18	6,00	3,54	0,93	a	
3: Somatic complaints	1,00	1,41	1,50	0,71	2	0,00	1,00	1,41	0,83	a	
4: Social problems	4,00	1,41	4,00	1,41	3,5	2,12	3,75	1,77	0,33	a	
5: Thought problems	5,50	2,12	5,50	2,12	3,75	1,06	2,75	1,06	0,02	a	
6: Attention problems	8,50	0,71	8,83	1,18	8,25	1,06	7,50	0,71	0,13	a	
7: Rule-breaking behavior	0,50	0,71	0,67	0,94	1,5	2,12	1,00	1,41	0,58	a	
8: Aggressive behavior	1,00	1,41	2,83	4,01	2	2,83	1,00	1,41	0,25	a	
9: Other problems	2,50	0,71	3,50	1,18	4,5	3,54	3,75	2,47	0,60	a	
Internalizing	11,00	0,00	9,33	2,83	10,50	1,41	8,50	4,24	0,47	a	
Externalizing	1,50	2,12	3,50	4,95	3,50	4,95	2,00	2,83	0,49	a	
1: Affective problems	3,50	0,71	3,00	1,89	3,50	0,00	2,00	0,71	0,26	a	
2: Anxiety problems	3,00	1,41	1,67	0,47	3,00	1,41	1,75	0,35	0,89	a	
3: Somatic problems	1,00	1,41	1,00	1,41	1,00	1,41	1,00	1,41	n.a.	a	
4: Attention deficit/Hyperactivity problems	4,00	1,41	4,67	2,36	4,75	0,35	4,75	1,06	0,48	a	
5: Oppositional/Defiant problems	4,00	1,41	4,67	2,36	4,50	0,71	4,75	1,06	0,48	a	
6: Conduct problems	0,50	0,71	0,67	0,94	1,50	2,12	1,00	1,41	0,58	a	
1: Sluggish cognitive tempo	2,50	0,71	3,17	0,24	2,50	1,41	2,25	0,35	0,05	a	
2: Obsessive- Compulsive problems	1,00	1,41	1,33	1,89	0,75	1,06	1,25	1,77	0,73	a	
3: Post-traumatic stress problems	6,00	0,00	5,67	0,47	7,25	1,77	5,00	0,00	0,65	a	

* p-value over the total study period and change from baseline

a Linear mixed model analysis

** baseline measurement of subject 1 is missing