Supplementary data

Title: Central adrenal insufficiency is rare in adults with Prader-Willi Syndrome

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Table S1. Results of the multiple-dose metyrapone test

Patient	Gender (M/F)[a]	Age (years)	BMI (kg/m^2)[b]	GH (Y/N)[c]	OAC/testosterone (Y/N)[d]	Baseline cortisol (nmol/L)	11-deoxycortisol (nmol/L)[e]	Delta ACTH (pmol/L)[f]
1	M	51·2	30·4	N	Y	235	407·9	6·1
2	M	37·8	48·1	N	Y	175	291·2	25·2
3	M	32·8	29·1	N	Y	350	520·2	41·3
4	M	20·1	41·9	N	Y	238	343·0	25·9
5	M	18·7	32·4	Y	Y	135	400·3	31·9
6	M	18·1	27·4	Y	Y	348	353·9	39·8
7	M	55·5	20·7	N	N	397	575·1	19·4
8	M	40·9	26·9	N	N	480	349·6	12·7
9	M	33·5	32·0	N	N	292	397·7	49·1
10	M	21·9	44·4	N	Y	252	279·9	21·9
11	M	27·6	26·3	N	Y	320	550·8	82·9
12	M	50·1	25·7	N	Y	395	390·9	15·8
13	M	42·9	25·0	N	N	301	355·0	10·9
14	M	35·7	24·6	N	Y	337	573·9	70·3
15	M	34·4	38·1	N	Y	428	358·3	43·8
16	M	29·2	27·8	N	Y	284	379·9	61·1
17	M	22·2	26·2	Y	Y	339	484·2	25·9
18	M	21·3	20·1	Y	Y	253	285·4	28·3
19	M	21·8	26·0	Y	Y	274	483·5	34·9
20	M	19·6	20·0	Y	Y	395	363·0	70·7
21	M	18·7	28·4	Y	Y	208	451·2	40·1
22	F	25·2	31·0	N	Y	709	660·7	13·2
23	F	21·8	34·4	N	N	126	495·3	40·2
24	F	20·9	36·9	Y	Y	508	425·3	13·7
25	F	29·9	37·7	N	Y	579	499·1	9·5

Patient	Gender (M/F)	Age (years)	BMI (kg/m^2)	GH (Y/N)	OAC/testosterone (Y/N)	Baseline cortisol (nmol/L)	11-deoxycortisol (nmol/L)	Delta ACTH (pmol/L)
26	F	29·7	26·8	Y	N	295	454·4	118·9
27	F	19·1	31·5	Y	Y	480	298·2	31·0
28	F	39·0	32·3	N	Y	233	544·3	73·6
29	F	29·1	32·8	Y	Y	345	500·3	71·0
30	F	27·6	33·9	Y	Y	167	476·2	4·1
31	F	22·5	21·7	Y	Y	601	487·9	44·0
32	F	22·3	27·2	N	Y	317	429·0	27·6
33	F	19·9	21·2	Y	Y	477	600·0	43·7
34	F	18·9	28·5	Y	Y	764	547·3	98·5
35	F	21·2	26·6	Y	Y	245	510·9	30·5
36	F	25·4	23·9	Y	Y	384	471·0	12·0
37	M	28·0	34·3	N	Y	306	391·2	-1·4
38	F	38·5	34·1	Y	N	216	247·8	20·5
39	F	29·5	31·5	N	Y	202	567·1	58·9
40	M	37·1	57·0	N	N	414	381·1	12·2
41	F	20·5	49·7	N	Y	508	569·4	91·9
42	M	21·4	20·6	Y	Y	273	401·7	28·2
43	M	51·1	29·4	N	N	350	334·0	39·9
44	M	19·4	24·7	Y	Y	331	694·0	61·5
45	M	22·9	25·6	Y	N	356	386·7	46·9
46	F	18·2	25·6	N	N	239	603·1	106·9

Y/N: yes/no. [a] M: male; F: female. [b] BMI: body mass index (kg/m^2). [c] GH: current growth hormone treatment. [d] OAC: current use of oral estrogen or progesterone; testosterone: current use of testosterone. [e] 11-deoxycortisol (nmol/L) during multiple-dose metyrapone test. Cut-off for central adrenal insufficiency is <230 nmol/L. [f] Increase in ACTH (pmol/L) level during multiple-dose metyrapone test.

Table S2. Results of the insulin tolerance test

Patient	Gender (M/F)[a]	Age (years)	BMI (kg/m^2)[b]	GH (Y/N)[c]	OAC/testosterone (Y/N)[d]	Baseline cortisol (nmol/L)	Peak cortisol (nmol/L)[e]	Glucose (mmol/L)	Delta ACTH (pmol/L)[f]
Dutch patients									
47	M	22·4	28·3	N	N	254	662	2·4	35·5
48	M	37·8	28·0	N	Y	306	734	1·9	28·1
49	M	32·1	21·8	N	Y	242	828	1·7	52·2
50	M	30·7	24·3	N	Y	153	552	1·4	90·5
51	M	26·3	28·7	N	N	165	687	1·7	44·4
52	M	25·7	29·1	Y	Y	119	532	1·9	63·4
53	F	47·3	40·1	N	Y	450	883	1·5	64·1
54	F	31·8	34·6	Y	N	133	778	2·1	61·5
55	F	55·3	21·8	N	N	502	717	1·9	21·2
56	M	34·8	28·0	N	Y	306	734	1·9	90·5
Swedish patients									
57	M	20·0	21·2	N	Y	177	632	2·6	NA
58	M	22·0	44·4	N	Y	181	822	1·6	NA
59	M	25·0	24·2	N	Y	194	502	1·7	NA
60	M	27·0	27·5	N	Y	265	703	1·9	NA
61	F	31·0	36·5	N	N	190	742	1·4	NA
62	M	36·0	32·7	N	Y	175	771	1·2	NA
French patients									
63	M	18·0	47·8	N	NA	229	665	1·6	NA
64	M	20·0	49·7	N	NA	235	563	2·0	NA
65	M	20·0	28·3	Y	NA	378	494	2·0	NA

Patient	Gender (M/F)	Age (years)	BMI (kg/m^2)	GH (Y/N)	OAC/testosterone (Y/N)	Baseline cortisol (nmol/L)	Peak cortisol (nmol/L)	Glucose (mmol/L)	Delta ACTH (pmol/L)
66	F	29·0	32·0	N	NA	229	944	1·5	NA
67	F	24·0	31·8	N	NA	102	712	1·6	NA
68	F	28·0	58·2	N	NA	166	759	1·7	NA
69	F	38·0	27·3	N	NA	384	1021	0·9	NA
70	F	23·0	20·3	N	NA	116	789	2·2	NA
71	F	19·0	43·6	N	NA	132	601	1·4	NA
72	F	18·0	24·0	N	NA	240	869	0·6	NA
British patients									
73	F	18·3	33·8	N	Y	327	817	2·1	NA
74	M	30·9	24·1	N	N	242	603	1·1	NA
75	F	19·3	26·9	Y	Y	166	535	1·4	NA
76	F	19·4	29·9	N	N	179	510	1·0	NA
77	F	20·6	30·7	N	Y	148	455	1·7	NA
78	M	23·7	34·9	Y	N	296	538	1·7	NA
79	M	19·3	24·8	N	N	93	502	1·5	NA
80	F	19·2	35·2	N	Y	545	971	1·8	NA
81	M	26·1	62·0	N	N	119	467	1·5	NA
82	M	24·7	57·2	N	N	120	496	1·4	NA

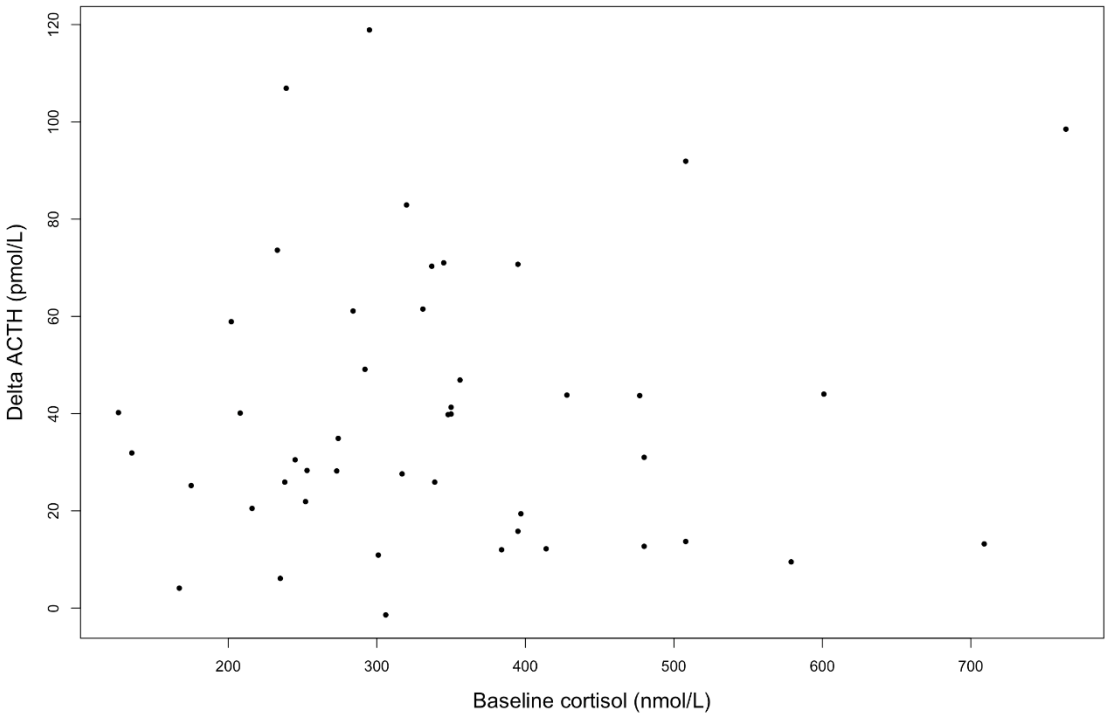
Y/N: yes/no. NA: Not available. [a] M: male; F: female. [b] BMI: body mass index (kg/m^2). [c] GH: current growth hormone treatment. [d] OAC: current use of oral estrogen or progesterone; testosterone: current use of testosterone. [e] Peak cortisol (nmol/L) level during insulin tolerance test. Cut-off for central adrenal insufficiency is <500 nmol/L (Dutch, French and Swedish patients) or <450 nmol/L (British patients). [f] Increase in ACTH (pmol/L) level during insulin tolerance test.

Table S3. Overview of adrenal function tests used to diagnose central adrenal insufficiency

Test	Test principle	Sensitivity (%)	Specificity (%)	Disadvantage
LDSST[a] [1]	Synthetic ACTH administration induces release of cortisol from the adrenal gland.	57	95	• Adequate for the diagnosis of primary adrenal insufficiency, but lack of consensus about use for the diagnosis of *central* adrenal insufficiency.
HDSST[b] [1]		61	95	
MTP[c] [2]	Metyrapone decreases cortisol production by inhibiting the 11ß-hydroxylase steroid enzyme. This stimulates ACTH production, leading to increased 11-deoxycortisol levels.	88-100	88-100	• Risk of acute adrenal insufficiency precipitated by metyrapone ingestion • Should be conducted on an inpatient basis.
ITT[d]	Insulin induces hypoglycaemia, which causes a release in ACTH and growth hormone (GH), leading to increased levels of cortisol.	Golden standard	Golden standard	• Risks associated with hypoglycaemia • Intravenous access at two different sites needed, which is impossible in many patients with PWS • Labour intensive, time consuming, unpleasant for patient

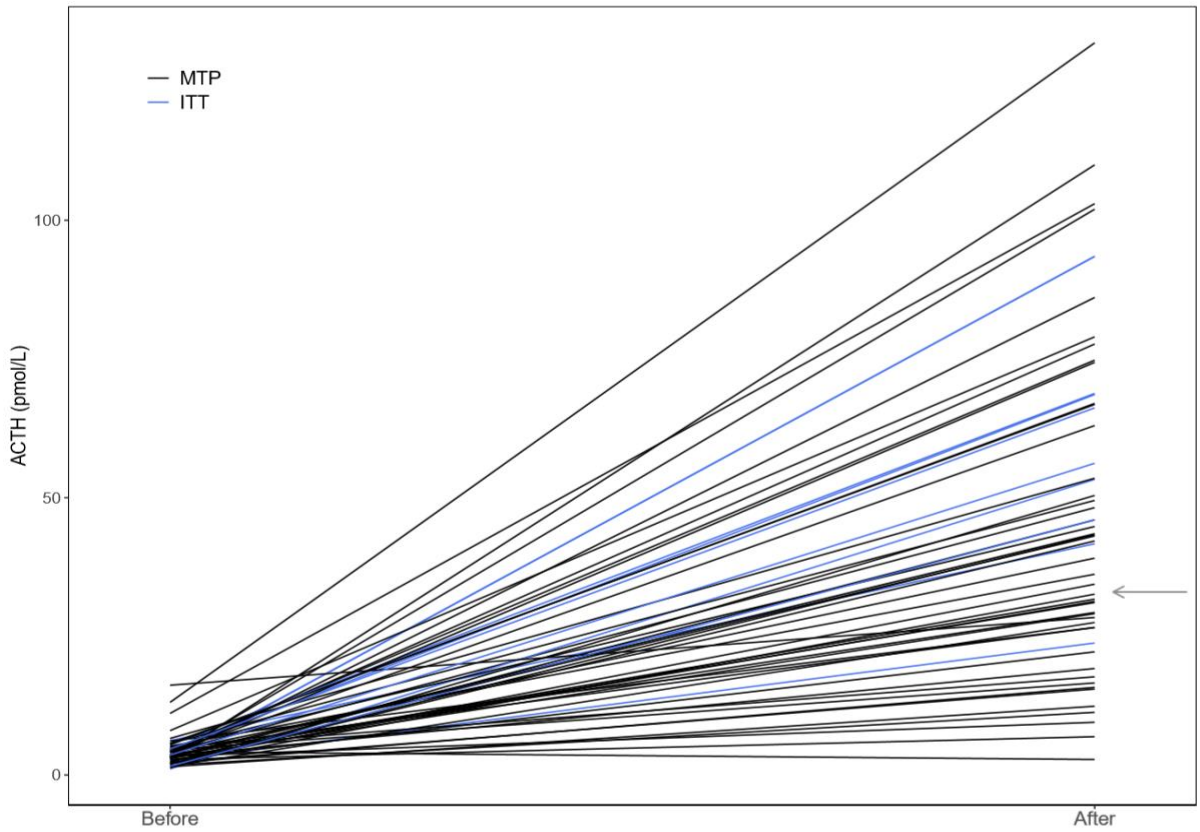
[a] LDSST: Low-dose short synacthen test. [b] HDSST: High-dose short synacthen test. [c] MTP: Metyrapone test. [d] ITT: Insulin tolerance test.

Figure S1. Relation between cortisol level (nmol/L) at baseline and increase in ACTH (pmol/L) during multiple-dose metyrapone test in patients with Prader-Willi syndrome



Relationship between baseline cortisol (nmol/L) and increase in ACTH (pmol/L) in Dutch patients with Prader-Willi syndrome who underwent multiple-dose metyrapone test. N = 46. Spearman's rho was 0·01 (P = 0·97).

Figure S2. ACTH levels (pmol/L) in Dutch patients with Prader-Willi syndrome who underwent the multiple-dose metyrapone test or insulin tolerance test



Spaghetti plot of the ACTH levels (pmol/L) in patients with Prader-Willi syndrome who underwent the multiple-dose metyrapone test (MTP; black line) or insulin tolerance test (ITT; blue line). For MTP test, n = 46. For ITT, n = 26. Median (range) ACTH level before and after metyrapone administration (MTP) were 3·5 (1·3 – 16·2) pmol/L and 37·7 (2·8 – 132·0) pmol/L. Median (range) ACTH level before and after insulin administration (ITT) were 3·3 (1·1 – 6·2) pmol/L and 61·2 (23·8 – 93·5) pmol/L. The grey arrow represents the ACTH cut-off value of 33 pmol/L, used for interpretation of the MTP in the Dutch pediatric study.[4] This would classify 21 patients with sufficient increase in 11-deoxycortisol levels as 'adrenal insufficient'.

References supplementary data

1. Dorin RI, Qualls CR, Crapo LM. Diagnosis of adrenal insufficiency. Ann Intern Med 2003;139:194-204.

2. Fiad TM, Kirby JM, Cunningham SK, McKenna TJ. The overnight single-dose metyrapone test is a simple and reliable index of the hypothalamic-pituitary-adrenal axis. Clin Endocrinol (Oxf) 1994;40:603-9.

3. Gibney J, Healy ML, Smith TP, McKenna TJ. A simple and cost-effective approach to assessment of pituitary adrenocorticotropin and growth hormone reserve: combined use of the overnight metyrapone test and insulin-like growth factor-I standard deviation scores. J Clin Endocrinol Metab 2008;93:3763-8.

4. de Lind van Wijngaarden RF, Otten BJ, Festen DA, et al. High prevalence of central adrenal insufficiency in patients with Prader-Willi syndrome. J Clin Endocrinol Metab 2008;93:1649-54.