Early testicular maturation is sensitive to depletion of spermatogonial pool in sickle cell disease

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Supplements

Supplemental Table 1. Testis volumes, hormonal parameters and histological parameters for patients with sickle cell disease included in this study.

	Program	Genetic	Age, years	Testis volume, mL	FSH, IU/L (1-7 IU/L)	LH, IU/L (2-10 IU/L)	T, nmol/L (>12 nmol/L)	Number of tubules	S/T	S/T Z-score	FI, %	FI Z-score
P1	A	HbS/ß+	2.8	0.3	0.8	<0.1	<0.1	182	3.5	4.1	96	7.6
P2	A	HbSS	3.9	1.0	0.5	Low	0.1	151	0.6	-4.3	33	-4.8
P3	A	HbSS	4.2	ND	ND	ND	ND	25	0.0	-13.2	0	-11.1
P4	A	HbSS	4.3	1.0*	0.4	0.1	0.1	67	0.5	-4.5	27	-6.0
P5	N	HbSS	4.5	ND	ND	ND	ND	44†	0.0	-15.2	0	-11.1
P6	N	HbSS	5.1	ND	ND	ND	ND	27†	0.0	-8.7	0	-11.8
P7	N	HbSS§	5.2	0.3‡	ND	ND	ND	169	0.1	-6.9	7	-10.4
P8	A	HbSS	5.8	0.5	0.3	Low	0.1	74	1.5	-1.0	65	0.5
P9	A	HbSS§	6.2	1.0	1.1	0.3	0.1	230	1.0	-4.5	50	-4.2
P10	A	HbSS	6.4	1.0	0.7	<0.1	<0.1	88	0.9	-4.7	43	-5.3
P11	N	HbSS	6.5	1.0‡	ND	ND	ND	25	0.0	-17.6	4	-12.1
P12	N	HbSS§	7.1	0.8‡	ND	ND	ND	44	0.5	-5.2	23	-10.4
P13	A	HbSS	7.1	1.0	0.5	Low	0.6	344	0.1	-12.6	3	-13.6
P14	A	HbSS	7.2	1.0	1.4	<0.1	<0.2	179	0.3	-6.7	23	-10.3
P15	A	HbSS§	7.3	1.0	0.1	Low	0.1	224	0.7	-4.1	39	-7.7
P16	N	HbSS	7.6	0.4‡	ND	ND	ND	21	1.1	-2.4	57	-4.7
P17	N	HbSS	7.9	1.0‡	ND	ND	ND	4	0.3	-7.5	25	-10.0
P18	A	HbSS	8.1	0.3	0.3	Low	Low	41	1.3	-3.1	68	-2.0
P19	A	HbSS	9.4	1.0	2.0‖	0.1	0.1	25	1.2	-1.2	40	-6.7
P20	A	HbSS	9.6	1.0	0.9	0.2	0.4	13	1.0	-1.6	62	-2.7
P21	A	HbSS	10.4	1.5	1.1	0.2	0.3	118	1.4	-1.8	63	-2.5
P22	A	HbSS	10.6	0.8	1.6	<0.1	<0.1	98	0.7	-2.8	43	-4.5
P23	A	HbSS	10.7	0.4	1.4	<0.1	<0.1	171	1.1	-2.2	51	-3.7
P24	A	HbS/ß°	11.2	0.6	1.0	<0.1	<0.1	220	1.5	-1.7	64	-2.4
P25	N	HbSS	11.5	ND	ND	ND	ND	112	1.9	-1.3	56	-3.1
P26	N	HbSS	13.1	ND	ND	ND	ND	7†	0.0	-5.3	0	-8.7
P27	N	HbSS	14.0	5.3‡	ND	ND	ND	29	3.6	-0.4	76	-1.2
P28	A	HbSS§	14.3	ND	4.6	2.0	12.7	14	0.9	-6.3	21	-11.9
P29	A	HbSS	15.1	2.6	1.3	2.3	<0.1¶	160	4.9	-1.0	87	-0.9
P30	CeRA	HbSS	21.5	11.0*	19.9‖	10.8‖	18.6	37	0.4	-42.5	5	NA
P31	CeRA	ND	48.5	9.5	15.4‖	3.8	13.0	42	10.14	-4.1	83	NA

Testicular volumes and hormone levels were determined shortly before the biopsy. Testicular volumes were measured using ultrasound unless indicated otherwise by a footnote. Hormone values were within the normal range unless indicated otherwise by a footnote. Hormone reference values for adult patients of the Centre of Reproductive Medicine and Andrology, Münster, are in agreement with common standards defined by the European Association of Urology,[1] for children to Konforte *et al.*[2]. For FSH, the reference values for boys are defined for the following age groups: 1- <5 years (< 0.91 IU/L), 5 -

<10 years (< 1.62 IU/L), 10- <13 years (0.35-3.91 IU/L), and 13 - <19 years (0.78-5.10 IU/L). For LH, the reference values are defined for boys of the following age groups: 1- <10 years (< 0.33 IU/L), 10 - <13 years (< 4.34 IU/L), 13 - <15 years (< 4.11 IU/L) and 15 - <17 years (0.79-4.76 IU/L). For testosterone, the reference values for boys of the following age groups are: 6 months- <9 years (< 1.24 nmol/L), 9 - <11 years (< 0.81 nmol/L), 11 - <14 years (< 15.4 nmol/L) and 14 - <16 years (1.25-21.9 nmol/L). A indicates Androprotect; CeRA, Centre of Reproductive Medicine and Andrology (Münster), FSH, follicle-stimulating hormone; LH, luteinizing hormone; low, minimal hormonal value (no exact determination possible by laboratory standard methods); NA, not applicable; N, Nordfertil; ND, not determined; and P, patient number.

*Testicular volume was measured with a Prader orchidometer.

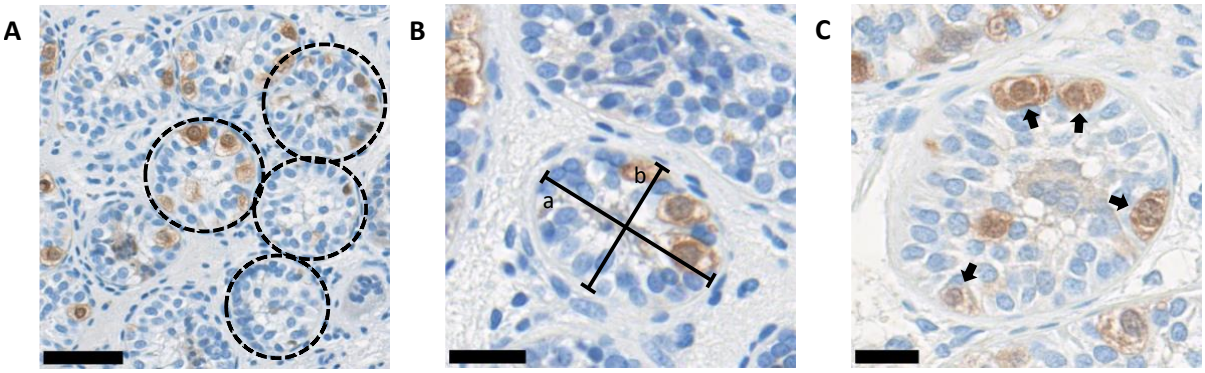
†Number of evaluated tubules following MAGEA4 stainings (P5: 19, P6: 1, P26: 0) were combined with results following DDX4 stainings (P5: 25, P6: 26, P26: 7).

‡Testicular volume was measured during the surgery with a ruler and calculated applying the following formula: Volume (mL) = 0.52 x longitudinal axis (cm) x transverse axis^2 (cm^2).

§Patient was also carrier of a mutation causing alpha thalassemia.

‖Hormone values were above the age-specific reference values.

¶Hormone values were below the age-specific reference values.



Supplemental Figure 1.

Representative micrographs of MAGEA4 stained testicular tissue specifying relevant criteria for inclusion of tubules and spermatogonia. (A) Tubules classified as round are marked by dashed lines. The scale bar represents 50 µm. (B) Tubules were considered as round if the ratio of the longer (a) to the smaller diameter (b) of the tubule was ≤ 1.5. The scale bar represents 20 µm. (C) Spermatogonia considered for quantitative analyses were identified based on MAGEA4 staining and localization along the basement membrane (indicated by arrow heads). The scale bar represents 20 µm.

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