Anti-Müllerian Hormone Deficiency in Females with Inherited Bone Marrow Failure Syndromes

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Outline

- Background
  - Fanconi Anemia (FA)
  - Dyskeratosis Congenita (DC)
  - Diamon-Blackfan Anemia (DBA)

- Females with Fanconi Anemia

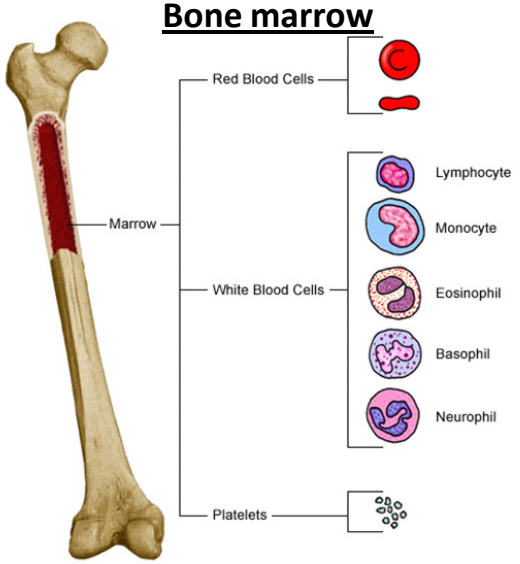
- Primary Ovarian Insufficiency (POI) & Anti-Müllerian Hormone (AMH)
  - What are they and how are they related
  - Can AMH serve as marker of POI in FA?

- Females with FA are deficient in AMH, what about DC and DBA?

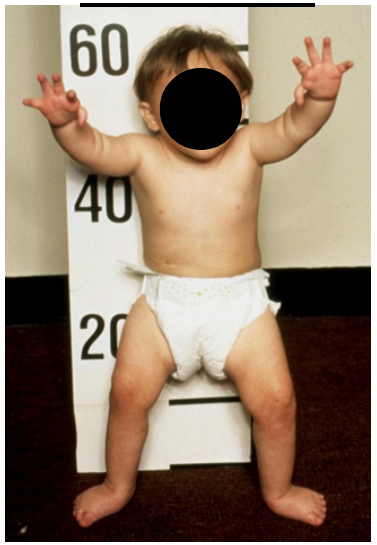
Fanconi Anemia (FA)

- Mutations in the FA/BRCA
  DNA Repair Pathway (>16 genes)
  - DNA repair defect
  - chromosome instability
- High risk of aplastic anemia
  - stem cell defect: deficiency in RBC, WBC, platelets
- High risk of cancer
  - leukemia
  - solid tumors
- Common physical abnormalities (60%):
  - short stature
  - thumb and radial malformations
  - other skeletal malformations
  - developmental delay
- Median age of survival: 29

Bone marrow



Patient with FA



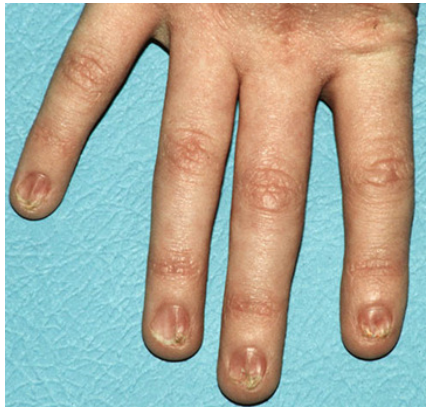
http://www.eskenazihealth.edu/layouts/MedTouch/Sublayouts/Modules/StayWell/GetImage.aspx?imageId=125422

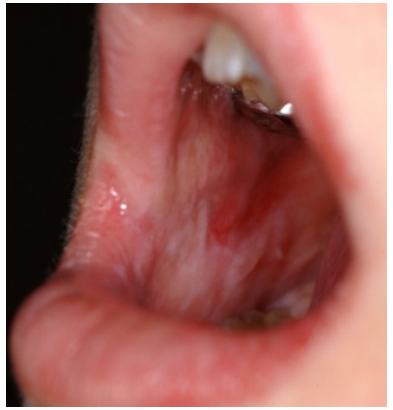
Shimamura A, Alter BP. *Blood Rev*. 2010.

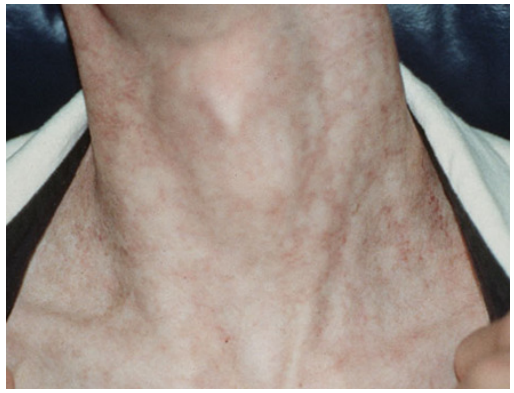
Dyskeratosis Congenita (DC)

- Mutations in telomerase and shelterin pathways (>9 genes)
  - very short telomeres
- High risk of aplastic anemia
- High risk of cancer
  - leukemia
  - solid tumors
- Common physical abnormalities (75%):
  - DC triad (46%):
    - dysplastic nails
    - lacy skin pigmentation
    - oral leukoplakia
  - developmental delay
  - short stature
  - skeletal malformations
- Median age of survival: 49

DC triad



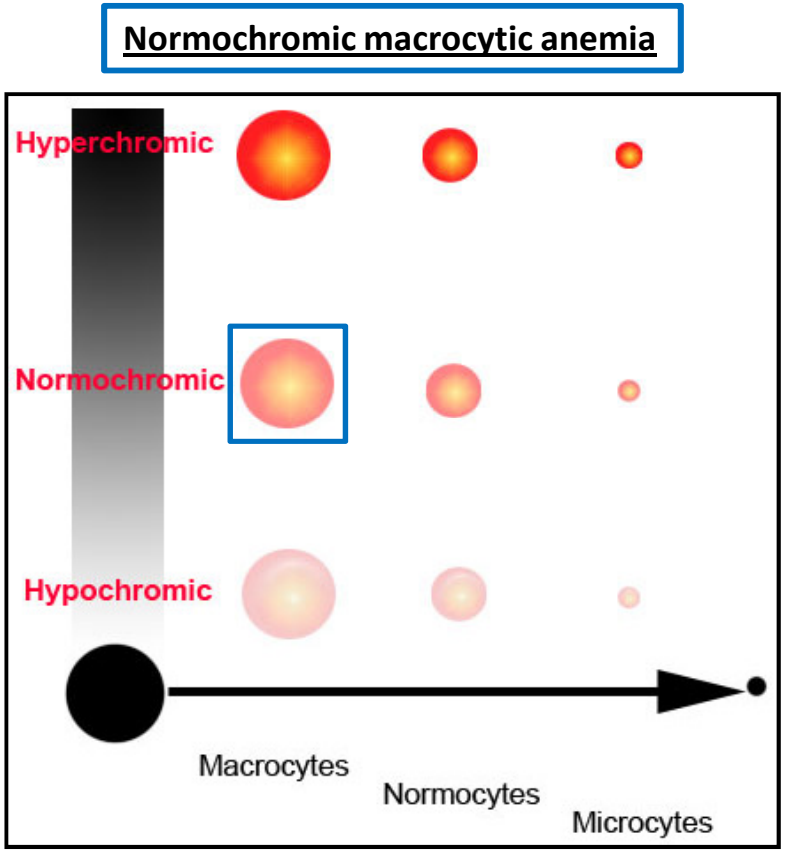




Shimamura A, Alter BP. *Blood Rev*. 2010.

Diamond-Blackfan Anemia (DBA)

- Mutations in genes encoding ribosomal subunits (>7 genes)
  - disruption of ribosomal biogenesis
  - activation of stress pathways (p53)
  - apoptosis of erythroid progenitors
- Anemia in infancy (90%)
  - normochromic, macrocytic
    - RBC deficiency
- Low risk of aplastic anemia
- Low risk of cancer
  - leukemia
  - sarcomas
- Common physical abnormalities (25%):
  - short stature
  - thumb malformations
  - cleft lip/palate
- Median age of survival: 40



Shimamura A, Alter BP. *Blood Rev*. 2010.
http://www.medicine.mcgill.ca/physio/vlab/bloodlab/mcv-mchc_n.htm

Focus on Fanconi Anemia (FA)

- FA is the most severe IBMFS
  - earliest age of onset of aplastic anemia
  - youngest median age for cancer-free survival: 29
    - most common FA cancers (relative risk):
      - acute myelogenous leukemia (AML) (600-fold)
      - head and neck (SCC) (500-fold)
      - cervical and vulvar SCC (3,000-fold)
- Compared to other IBMFS, females with FA had:
  - a higher rate of irregular menses
  - a higher rate of infertility
  - lower rates of pregnancy
  - a higher rate of gynecological neoplasia
  - a higher rate of primary ovarian insufficiency (POI)

Stratton P, Giri N, Alter BP. Society for Gynecologic Investigation Meeting, March 2010.
Shimamura A, Alter BP. *Blood Rev*. 2010. SEER data

Primary Ovarian Insufficiency (POI)

- Definition of POI

When at least one of the following occurs prior to age 40

  - establishment of a suboptimal follicular pool
  - follicular dysfunction
  - accelerated depletion of the follicular pool

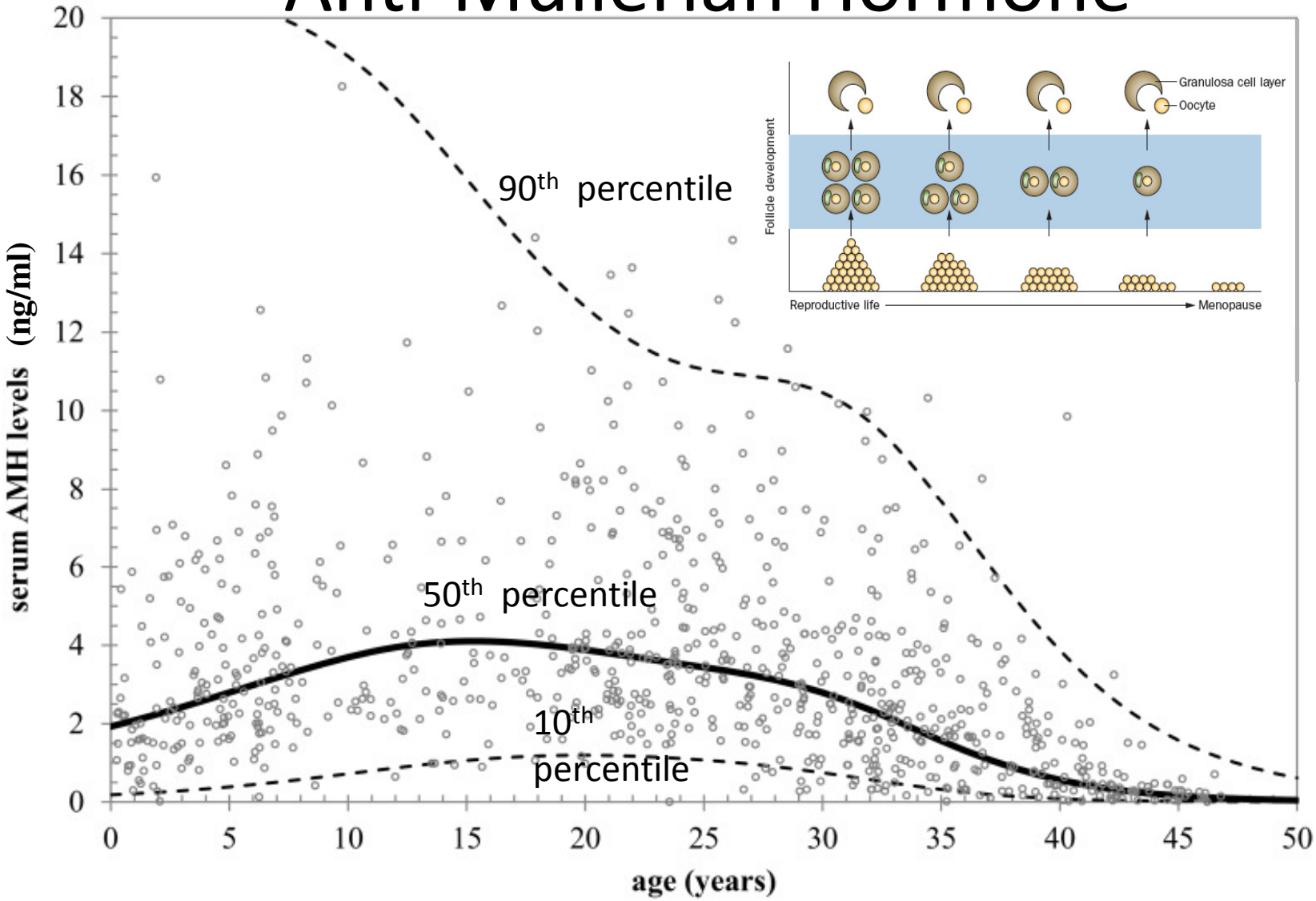
- Diagnosis of POI
  - 2 elevated measures of follicle-stimulating hormone (FSH)
  - amenorrhea for more than 4 months

- Marker of POI

Anti-Müllerian hormone (AMH) has been shown to be a better marker of diminished ovarian reserve/POI compared with FSH

Welt CK. *Clin Endocrinol (Oxf)*. 2008
Nelson LM. *N Engl J Med*. 2009
Kunt C, et. al. *Arch Gynecol Obstet*. 2011

Anti-Müllerian Hormone

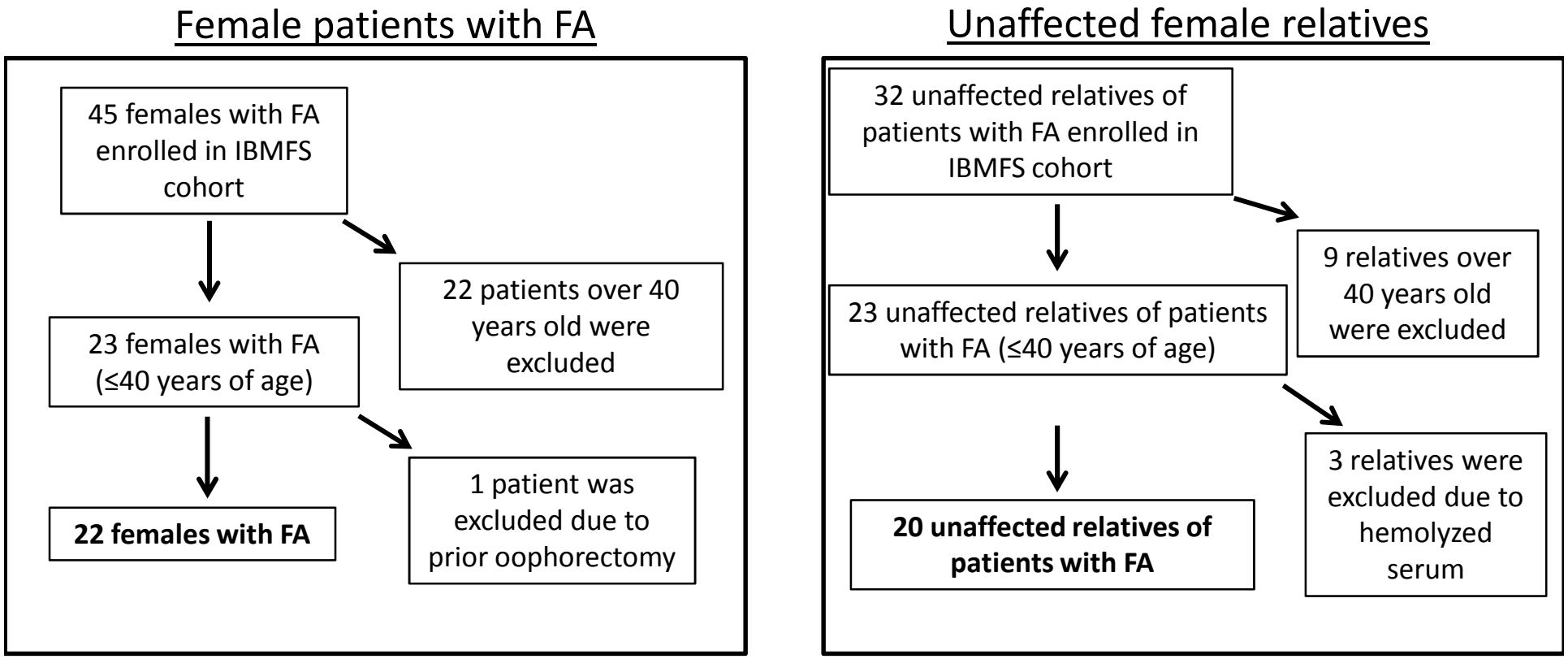


- AMH is a peptide hormone within the TGF-beta family of growth factors that is circulated in the blood
- AMH is produced exclusively in the granulosa cells within the ovaries
- AMH levels do not significantly fluctuate during the menstrual cycle

Visser JA, et. al. *Nat. Rev. Endocrinol.* 2012
Lie Fong S, et. al. *J. Clin. Endocrinol. Metab*. 2012

Can AMH serve as a cycle-independent marker of POI in female FA patients?

Serum sample selection: NCI natural history study of IBMFS



AMH was measured using an AMH ELISA (Beckman Coulter)

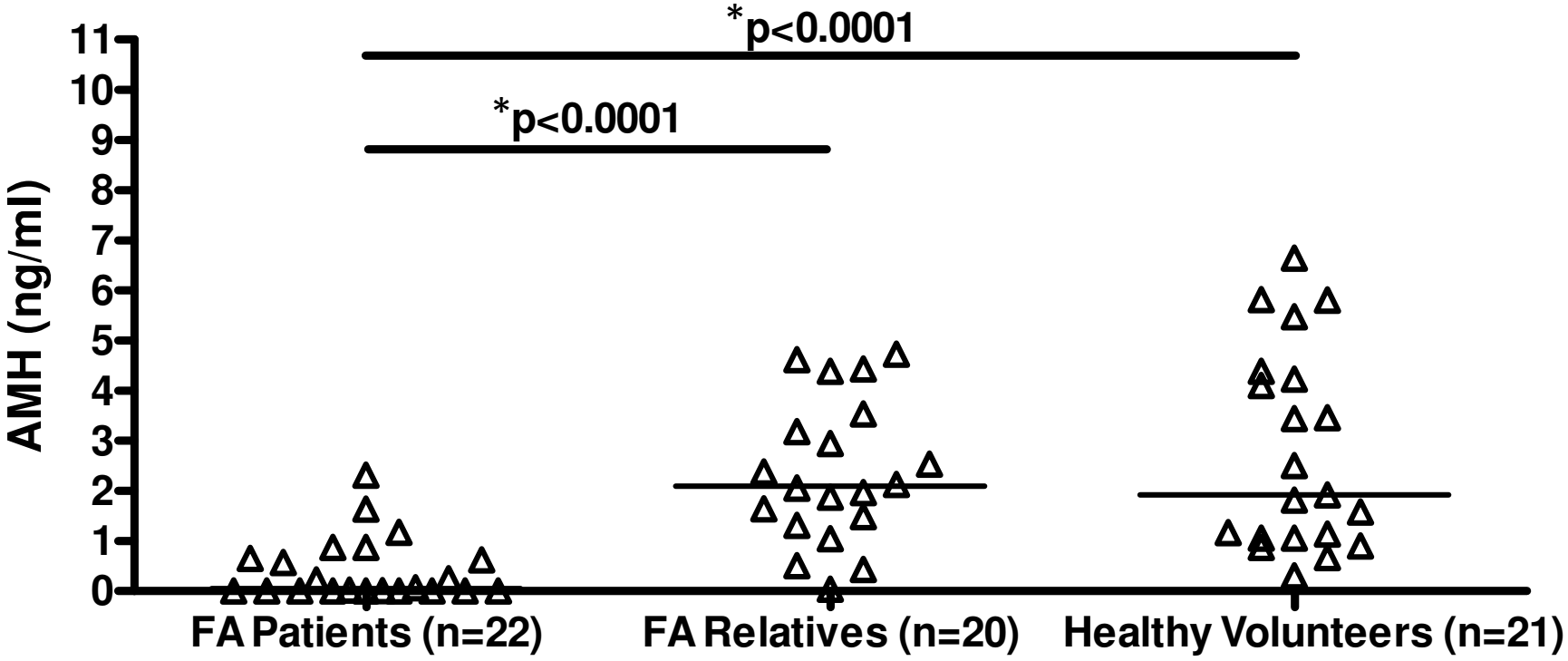
Healthy volunteers (n=21): OHS healthy donor program, Dr. Lauren Wood, Equitech

Study participants

Age and Menarchal Parameters	FA patients	FA relatives	Unrelated controls	P value
Number of subjects	22	22	21	-
Median age when serum drawn (range)	14.5 (7-37)	33.5 (3-40)	27 (12-40)	0.004
Median age at menarche (range)	13.5 (11-17)	12.5 (8-15)	NA	0.09
Number of subjects over the age of 10/pubertal+	15	18	21	0.37

Parameter	N	Clinical details in the 15 females with FA over 10 years of age
POI	7	4 = ↑ FSH; 3 = menopausal symptoms
Genes	15	11 = *FANCA*; 4 = *FANCC*
Cancers	6	1 each: skin, vulvar/anocervical, breast, scalp, esophageal, recurrent perianal/finger

FA patients have significantly lower levels of AMH when compared to unaffected relatives and healthy volunteers



Sklavos MM, Giri N, Stratton P, Alter BP, Pinto LA. *J Clin Endocrinol Metab*. 2014 Jan 17:jc20133559.

*Mann Whitney Test

Impact

- Most females with FA fail to produce normal levels of AMH at anytime in their lives

- Ovarian defects are a common factor in the otherwise heterogeneous clinical disease

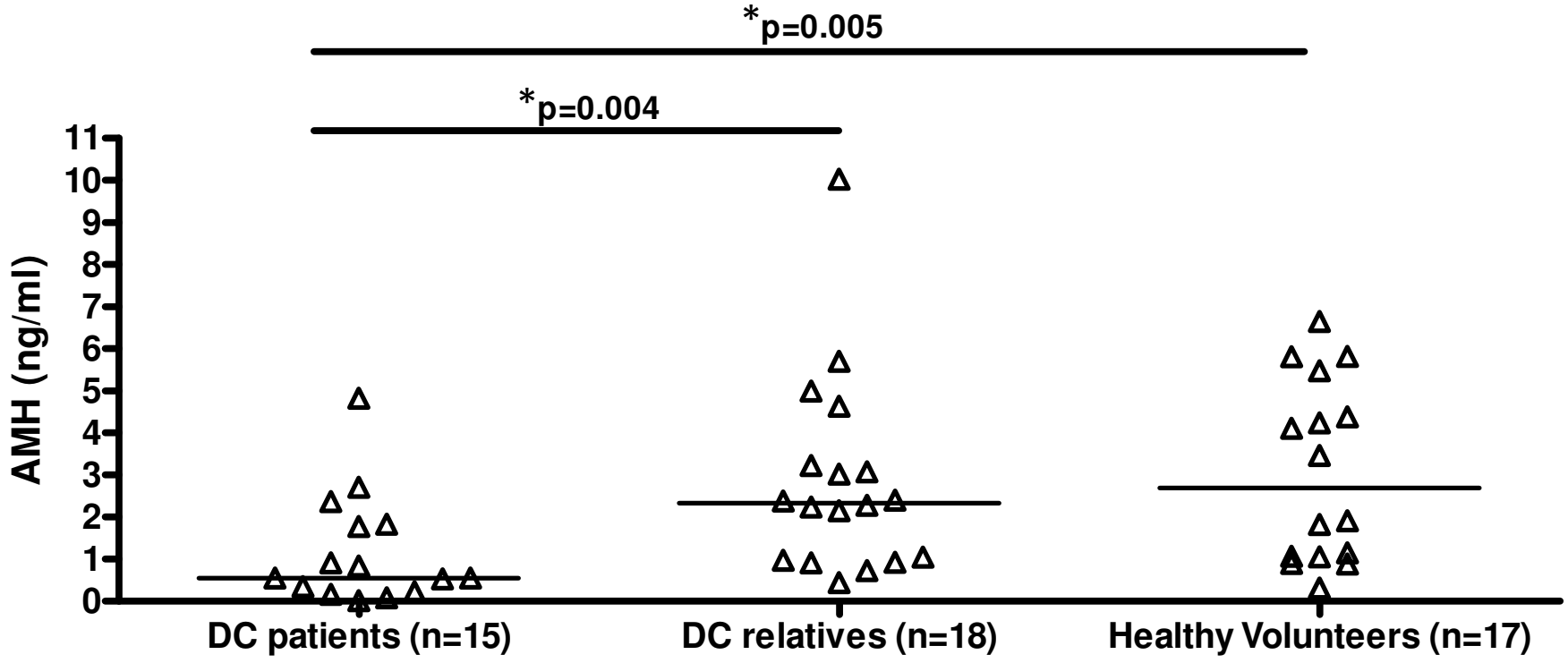
- Test AMH at FA diagnosis and monitor levels throughout life
  - Prophylactic management of complications associated with POI
    - infertility
    - osteoporosis
    - menopausal symptoms

- Perhaps different mutations within *FANC* genes may be associated with the severity of AMH deficiency in patients with FA

- Preclinical research has demonstrated anti-cancer properties of AMH warranting further research to determine whether AMH deficiency contributes to increased cancer risk

What about AMH levels in DC and DBA?

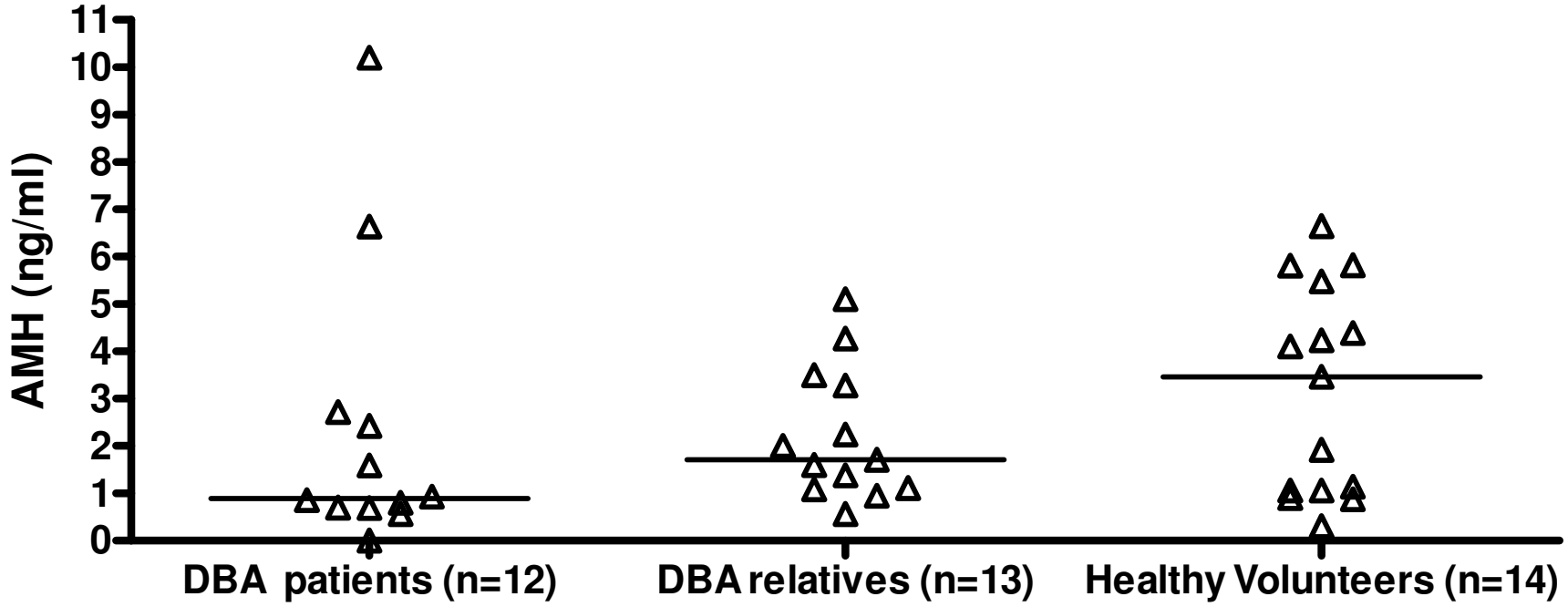
DC patients have significantly lower levels of AMH when compared to unaffected relatives and healthy volunteers



median age (range):	16 (4-30)	22.5 (2-40)	24.5 (12-31)

*Mann Whitney Test

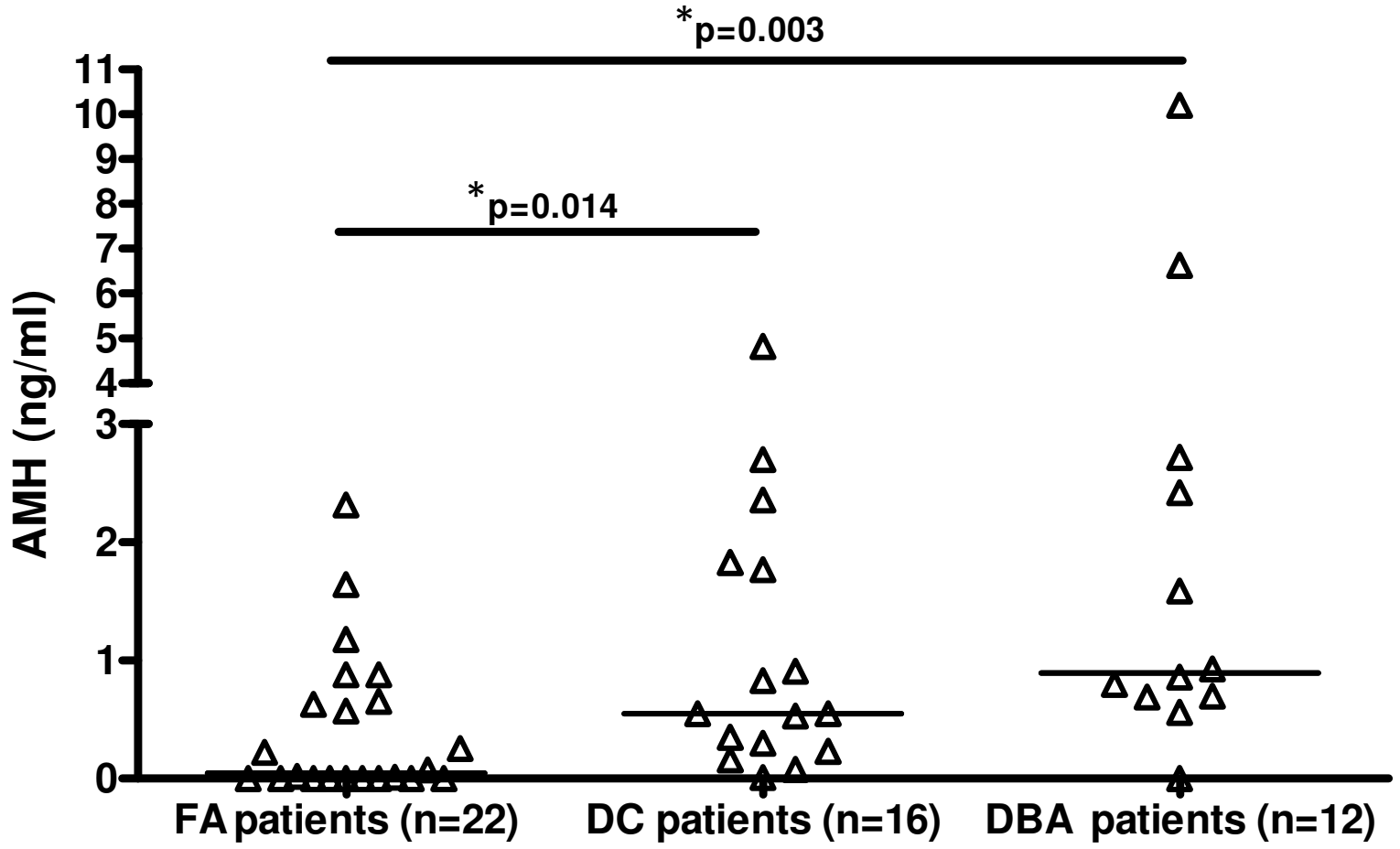
DBA patients show a trend for lower AMH levels



median age (range):	15.5 (1-30)	11 (1-34)	24 (12-29)

Mann Whitney Test

AMH levels are significantly lower in FA females



median age (range):	14.5 (7-37)	16 (4-30)	15.5 (1-30)

*Mann Whitney Test

Conclusions

- Females with FA and DC have significantly lower levels of AMH compared with unaffected relatives or healthy volunteers
- Females with FA have significantly lower levels of AMH compared with females with DC or DBA
- AMH levels appear to follow the inverse trend of disease severity and cancer incidence:

	FA	DC	DBA
AMH levels	↓↓↓	↓↓	↓
Disease severity	↑↑↑	↑↑	↑
Cancer incidence	↑↑↑	↑↑	↑

What's Next?

- On-going AMH studies
  - AMH levels in males with IBMFS
  - AMH and cervical cancer risk (Nico Wentzensen, SUCCEED)
  - AMH levels in relation to other hormone measures in 18-90 year old healthy males (Britton Trabert & Katherine McGlynn, NHANES)

Acknowledgements

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  - Gloriana Shelton
  - Marcus Williams

- Allan Hildesheim, PhD

- The patients and families participating in the NCI IBMFS Cohort

- NCI-Frederick Occupational Health and Safety Staff
- Healthy volunteers from the NCI-Frederick Healthy Donor Program
- Lauren Wood, MD
- Mark Greene, MD
- Lisa Leathwood
- Maureen Risch

Inherited Bone Marrow Failure Syndromes:

- Fanconi Anemia (FA)
  - mutations in the FA/BRCA DNA Repair Pathway
  - high risk of aplastic anemia and cancer
  - median age of survival: 29

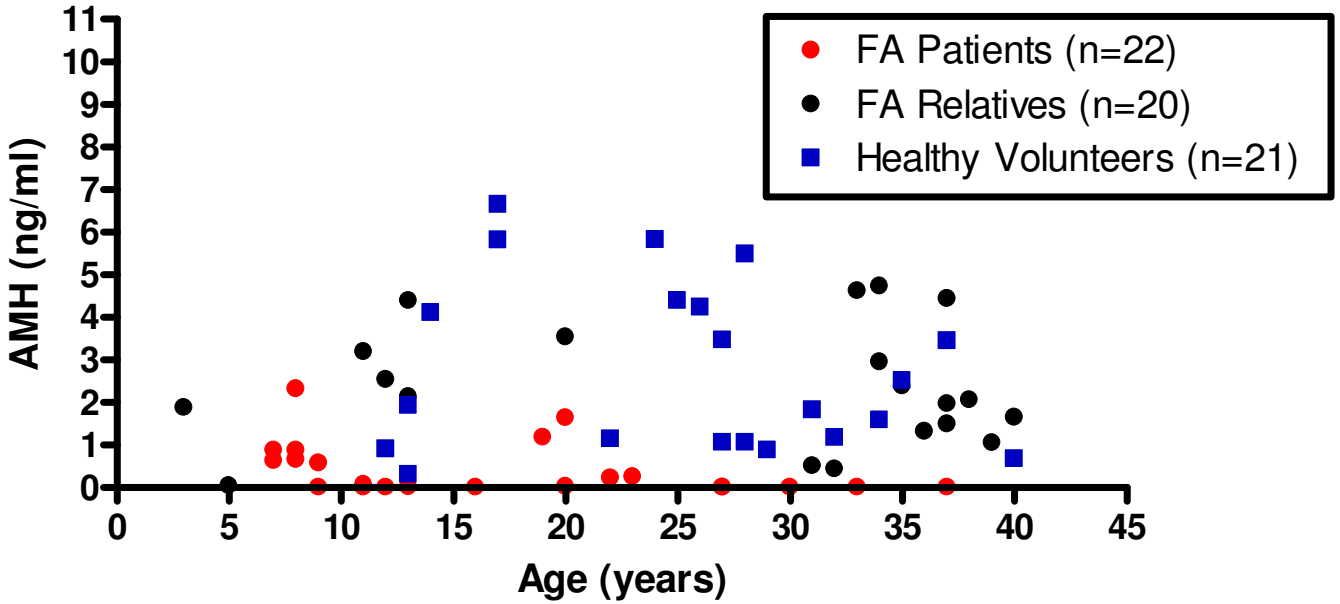
- Dyskeratosis Congenita (DC)
  - mutations in genes involved in telomere maintenance
  - high risk of aplastic anemia and cancer (later onset vs. FA)
  - median age of survival: 49

- Diamond-Blackfan Anemia (DBA)
  - mutations in genes encoding ribosomal subunits
  - low risk of aplastic anemia and cancer; anemic at birth or shortly thereafter
  - median age of survival: 40

- Causes of death a result of complications from:
  - bone marrow failure
  - bone marrow transplant
  - cancer

Shimamura A, Alter BP**.** *Blood Rev*. 2010.

AMH Levels: Patients with FA, unaffected relatives, healthy volunteers



AMH and cancer

- AMH has been shown to inhibit cell growth and metastasis in human breast, ovarian, endometrial, and cervical cancer cell lines and tumor development in mouse models of ovarian cancer

- Genetic mutations and inactivation within the FA/BRCA DNA repair pathway in the general population also result in increased risks of cancers of the cervix, head and neck, ovary, and breast

- Studies have demonstrated that female patients with Hodgkin and non-Hodgkin lymphoma (HL, NHL) and female patients with HL and other forms of childhood cancer (ALL, AML, etc) have significantly decreased AMH levels compared to healthy controls

MacLaughlin DT, Donahoe PK. *Future Oncol*. 2010.
Titus S, et.al. *Sci Transl Med*. 2013.

Lawrenz B, et.al. *Fertil Steril.* 2012.
van Dorp W, et. al. *Hum Reprod.* 2013.

To compare the gynecologic natural history in women with IBMFS

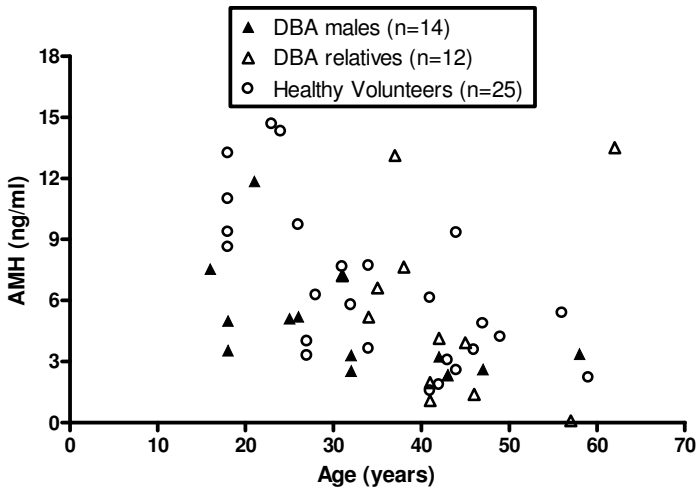
- Women with FA were compared with those with DC and DBA in the NCI natural history study of IBMFS

- All women >age 10 were included and were evaluated at similar median ages

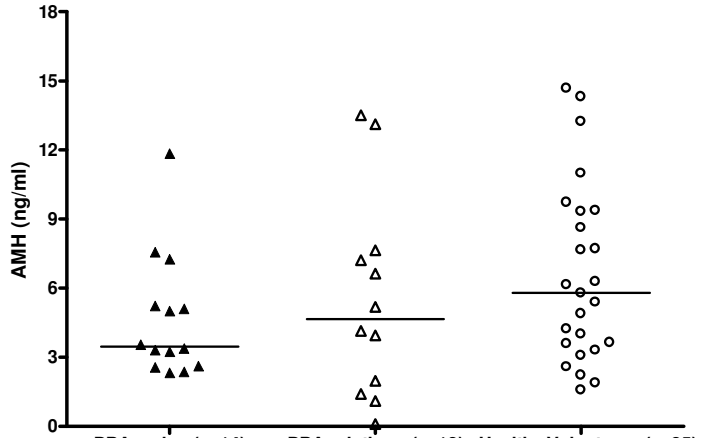
- 61 women:
  - 32 with FA
  - 15 with DC
  - 14 with DBA

Stratton P, Giri N, and Alter BP. Society for Gynecologic Investigation, Orlando Florida, March 2010.

DBA males

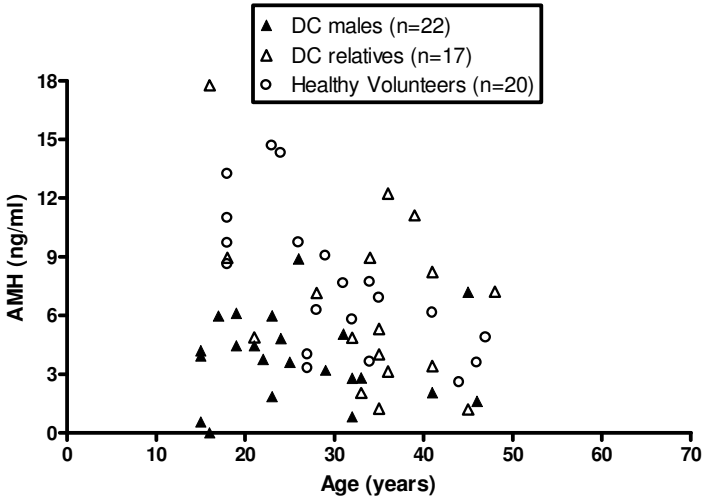


AMH levels: DBA patients, unaffected relatives, and healthy volunteers

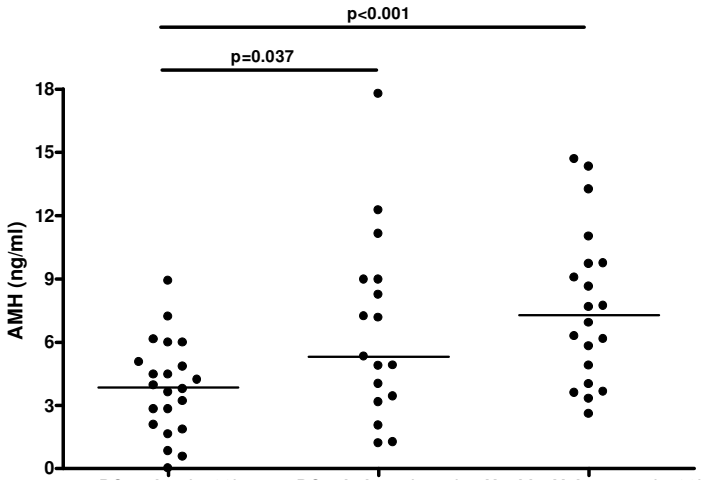


median age (range):	DBA males (n=14)	DBA relatives (n=12)	Healthy Volunteers (n=25)
	31.5 (16-58)	41 (31-62)	34 (18-59)

DC males



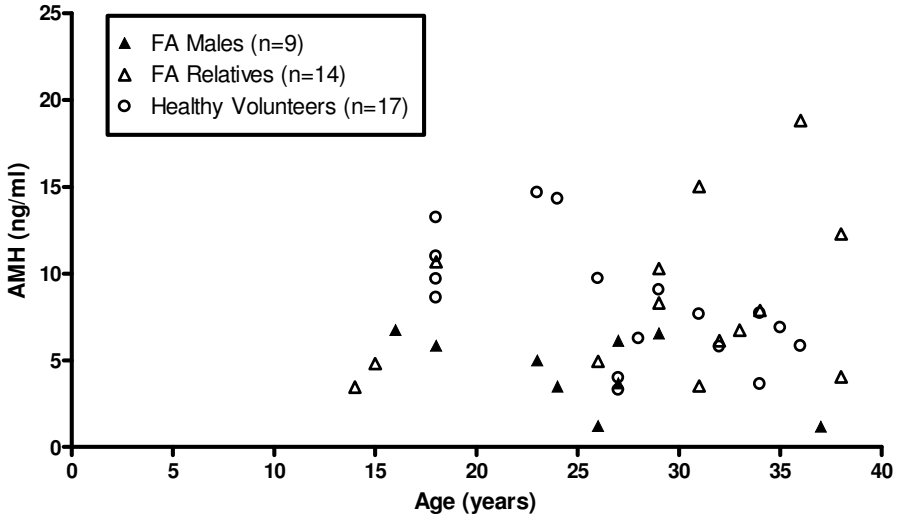
AMH levels: DC patients, unaffected relatives, and healthy volunteers



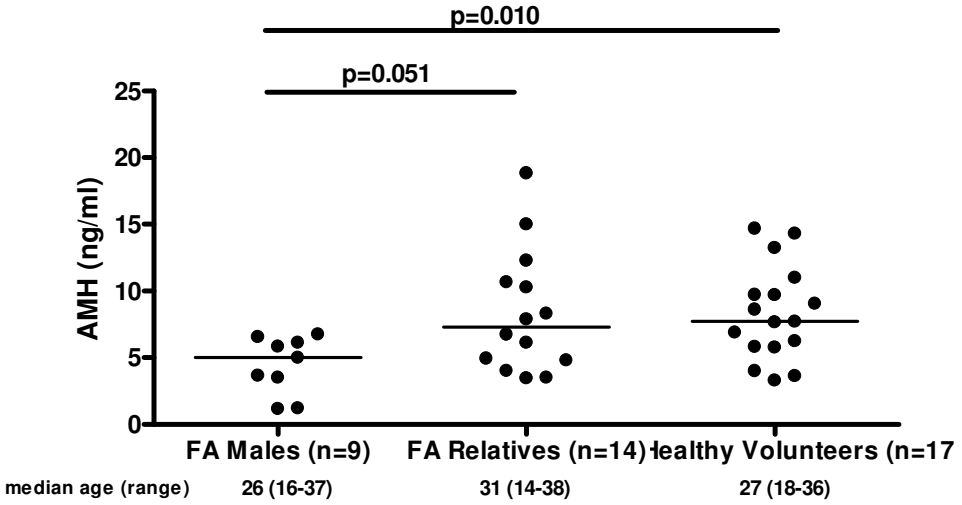
median age (range):	DC males (n=22)	DC relatives (n=17)	Healthy Volunteers (n=20)
	23.5 (15-46)	35 (18-48)	28.5 (18-47)

Statistically significant difference (p=0.004) between median ages of DC males and DC relatives

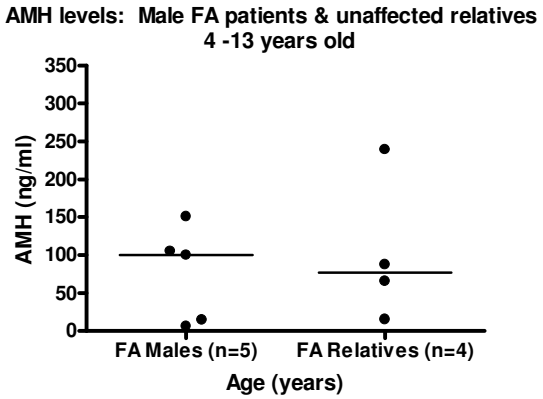
FA males

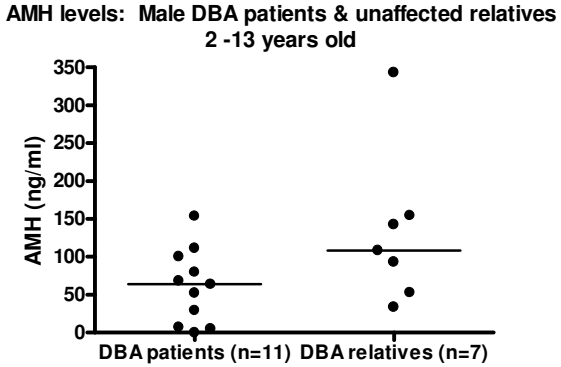


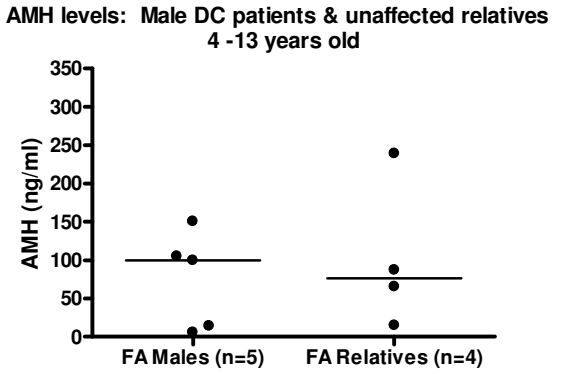
AMH levels: FA patients, unaffected relatives, and healthy volunteers

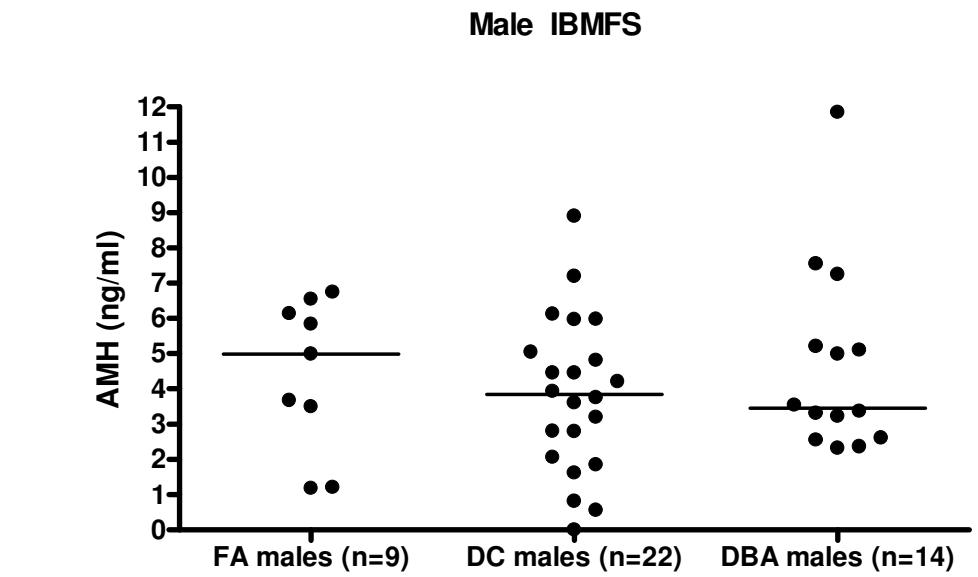


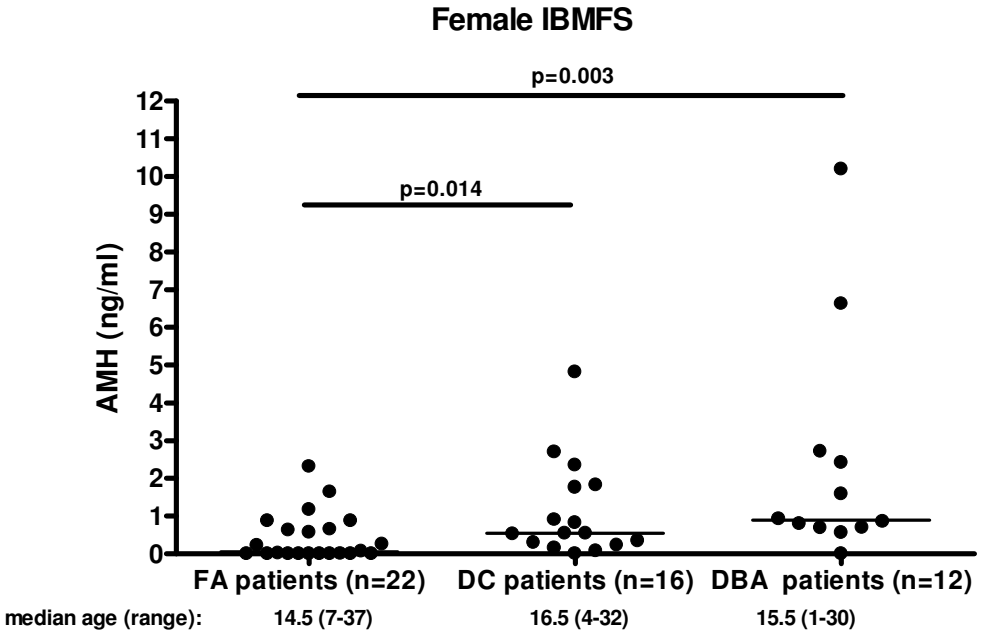
IBMFS male patients and relatives 0-13 years old







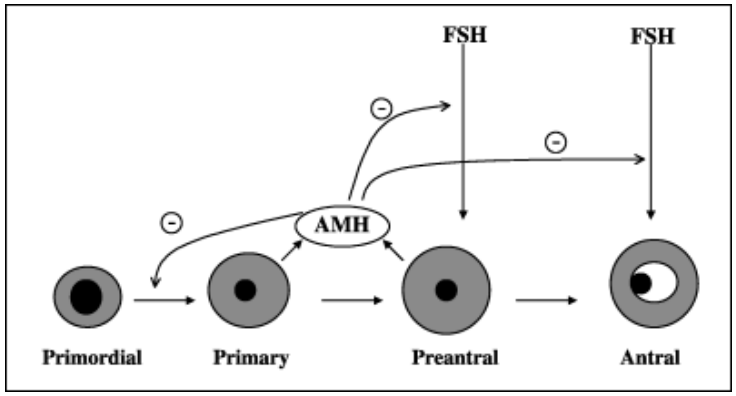

Male IBMFS
AMH (ng/ml)
12
11
10
9
8
7
6
5
4
3
2
1
0
FA males (n=9)
DC males (n=22)
DBA males (n=14)
median age (range):
26 (16-37)
23.5 (15-46)
31.5 (16-58)


Female IBMFS
p=0.003
p=0.014
AMH (ng/ml)
12
11
10
9
8
7
6
5
4
3
2
1
0
FA patients (n=22)
DC patients (n=16)
DBA patients (n=12)
median age (range):
14.5 (7-37)
16.5 (4-32)
15.5 (1-30)

Diagnosis:

- FA: present with physical anomalies, anemia, or malignancy at young age
  - confirmed by chromosomal aberrations in blood lymphocytes cultured with a DNA-crosslinking agent
  - Complementation group determined by correction of FA cellular phenotype by retroviral transfection of lymphoblasts or fibroblasts with one of known FA (*FANC*) genes
- DC: present with features of DC or other findings +/- anemia or cancer
  - Detection of very short telomeres in blood leukocytes
  - Identify mutations in DC genes (*TERT, TERC, TINF2*)
- DBA:

Anti-Müllerian hormone (AMH) in female reproduction: is measurement of circulating AMH a useful tool?



The activation of primordial follicles and the pace of follicular development are regulated by both positive and negative factors. AMH is considered as a negative regulator of the early stages of follicular development (Fig. 1). Homozygous AMH knockout female mice appeared normal.[6] However, careful analysis showed that homozygous knockout females have more growing preantral and small antral follicles than wild-type mice when they are prepubertal and as young adults.[24] However, their stock of primordial follicles becomes depleted earlier in life. Heterozygotes are intermediate between mutant and wild-type ovaries.

Clinical Endocrinology
Volume 64, Issue 6, pages 603-610, 5 MAY 2006 DOI: 10.1111/j.1365-2265.2006.02533.x
http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2265.2006.02533.x/full#f1

Females 9 and younger with Fanconi Anemia

TA (Need UPN)	Age at study	Age at menarche	Menses	Hormonal therapy	Tanner stage	AMH (ng/ml)	Number and d escription of anomalies	FA Gene	BMT prior to study	Prevalent Cancer	Other conditions
NCI-306-1	7	NA	NA		br 1/pubic 1	0.63	4, Short stature, microcephaly, abnormal thumb, deafness	*FANCA*	no	N/A	
NCI-120-1	8	NA	NA		br 1/pubic 1	2.316	0, normal	*FANCC*	no	N/A	BMT after study
NCI-221-1	8	NA	NA		br 2/pubic 2	0.653	6, absent thumb, abnormal radius, absent/abnormal kidney, congenital dislocation of hips, scoliosis	Unknown	no	N/A	scoliosis
NCI-332-1	8	NA	NA	growth hormone	br 1/pubic 1	0.88	5, short stature, microcephaly, developmental delay, deafness, cardiac anomaly, esophageal atresia, valophalangeal incompetence	*FANCA*	no	N/A	Valophal incompetence, esophogeal atresia
NCI-8-1	9	NA	NA	thyroid replacement	br 1/pubic 1	0	9, short stature, microcephaly, developmental delay, absent/abnormal thumb, and radius, deafness, abnormal kidney, cardiac anomaly, anal atresia, esophageal atresia, valophalangeal incompetence	*FANCJ*	no	N/A	anal atresia, Valophal incompetence, esophogeal atresia
NCI-72-1	9	NA	NA		no signs	0.573	1, developmental delay	*FANCF*	no	N/A	BMT after study

Females 10 and older with Fanconi Anemia

UPN	Age at study	Age at menarche	Menses	Hormonal therapy	Seeking fertility	POI	AMH (ng/ml)	Number of anomalies	FA Gene	FA mosaic	BMT prior to study	Age at BMT	Time since BMT (years)	Prevalent Cancer	Incident/Recurrent Cancer
NCI-213-1	11	NA	NA	estradiol	no	yes	0	3	*FANCA*	no	yes	9	1	no	no
NCI-246-1	11	NA	NA	oxymetholone	no	no	0.07	2	*FANCA*	no	no			no	no
NCI-25-1	12	NA	NA	premarin	no	no	0	8	*FANCC*	no	yes	6	6	no	no
NCI-98-1	14	11	irregular		no	no	0.02	6	*FANCA*	no	yes	8	6	no	no
NCI-59-1	16	14	regular		no	no	0	0	*FANCA*	no	no			no	no
NCI-111-1	19	15	regular		no	no	1.18	0	*FANCA*	no	no			no	no
NCI-12-1	20	14	irregular		no	no	0.02	0	*FANCA*	no	no			no	no
NCI-331-1	20	12	regular		no	no	1.64	0	*FANCA*	no	no			no	no
NCI-19-1	22	17	irregular[a]	danazol	no	no	0.23	3	*FANCC*	no	no				basal cell skin
NCI-169-1	23	13	irregular		no	yes	0.26	3	*FANCC*	no	yes	7	16		vulvar, anocervical
NCI-73-1	27	13	irregular		yes	yes	0	4	*FANCA*	yes	no				breast
NCI-73-2	27	13	irregular		yes	yes	0	7	*FANCA*	yes	no			no	no
NCI-33-1	30	12	irregular	provera, estrogen patch	no	yes	0	8	*FANCC*	no	yes	10	20	vulvar, tongue	scalp
NCI-61-1	33	14	irregular	combipatch	yes	yes	0	5	*FANCA*	yes	no			tongue, skin	esophageal
NCI-144-1	37	14	irregular	drospirenone ethinyl estradiol, nandronolone	no	yes	0	1	*FANCA*	no	no			vulvar with perianal spread	recurrent perianal, finger

Females with Dyskeratosis Congenita

TA (need UPN)	Age at study	Age at menarche	Menses	Hormonal therapy	Sexually Active	Pregnancy	POI	AMH (ng/ml)	Number of anomalies	BMT prior to study	Cancer	Other Conditions
TA 2637	4	NA	NA	NO	N/A	N/A		2.36		No		
TA 3096	6	NA	NA	NO	N/A	N/A		2.7	Mild development impairment		no	
TA 1710	8	NA	NA	NO	N/A	N/A		0.35	2, microcephaly, dev delay. DC triad	No	no	
TA 3428	9	NA	NA	NO	N/A	N/A		0.83	Microcephaly, DC triad, esophageal stricture, learning difficulty	No	no	
TA 2483	9	NA	NA	NO	N/A	N/A		0.01	DC triad	Yes, 5 years prior	no	
TA 2053	13	14	NA	NO	N/A	G1P1 (16 yo)		1.83	microcephaly	No	no	
TA 1049	15	11	Menorrhagia	Ogesteral to prevent menses	yes	No -OCP		0.23	DC triad	No	no	RBC/Pl tx/ Iron overload
TA 2938	16	?	No details	No details	No details	no		0.53	DC triad, bone fractures, Coats retinopathy	No	no	
TA 1649	17	13	heavy	D-provera, Anadrol	yes	No - contraception		0.16	Developmental delay	No	no	RBC tx / Iron overload
TA 3051	18	13	Normal	NO	no	No		0.08	Dev delay, microcephaly, DC-HH variant.	No	No	
TA 3025	20	12	Normal	Oral contraception	yes	No – OCP		0.91	No details	No	NO	
TA 3566	26	12	Normal	Oral contraception	yes	No – OCP		1.77	none	No	NO	
TA 0562	27	14	Irregular/heavy	Anadrol	yes	G2P2 (4 yr later)		4.82	DC triad	No	NO	Pregnancy w PGD/IVF after BMT
TA 2996	28	17	irregular		yes	fertility issues – due to multiple medical problems		0.55	No physical anomaly – skin cancer, MDS/AML	No	SCC skin age 16. AML.	Ovarian US for fertility issue showed nl ovaries
TA 1310	30	14	Normal	No	yes	G2P2		0.55	none	No	NO	

Females with Diamond-Blackfan Anemia

TA (need UPN)	Age at study	Age at menarche	Menses	Hormonal therapy	Sexually Activity	Pregnancy	POI	AMH (ng/ml)	Number of anomalies	BMT prior to study	Cancer	Other Conditions
TA 2499	1	N/A	N/A	Prednisone	NA	N/A		0.69	0	No	No	
TA 3499	2	N/A	N/A		NA	N/A		0.7	0	No	No	
TA 0458	13	N/A	N/A	Prednisone; Lupron	NO	N/A		0.56	1, short stature	No	No	Lupron to delay epiphyseal fusion and thus promote growth
TA 3394	14	14	Normal	no	NO	0		0.93	0	No	No	Nl pubertal dev. On RBC tx/Exjade. Has iron overload
TA 3430	14	No details	No details		No details	0		0.86	0	No	No	
TA 2905	15	13	Heavy, at Regular intervals	Prednisone	yes	0 (condom)		1.59	1, short stature	No	No	Prednisone responsive
TA 2998	16	13	regular	no	Don't know	0		2.72	0	No	No	Iron overload/ RBC tx-dependent
TA 3429	17	No details	No details	Prednisone on and off	Don't know	0		6.63	0	No	No	
TA 2908	20	14	Irregular w cramps	No	yes	0 – on OCP		10.2	0 – silent carrier	No	No	
TA 0681	21	13	Regular	No	Yes - Lesbian	0		2.42	0 – silent carrier	No	No	
TA 0410	28	13	irregular	Prednisone on and off. HRT for ovarian insufficiency	yes	0	yes	0	1, short stature	No	No	Iron overload induced endocrinopathies, ovarian insufficiency, osteoporosis
TA 0691	30	13*	hormonally induced cycling.	HRT	yes	0	yes	0.8	1, absent/abnormal kidney	No	No	Iron overload induced endocrinopathies. small uterus, hypothyroid, diabetes