Introduction and overview of sex differences in MS risk and course:

Tanuja Chitnis MD

Associate Professor in Neurology, HMS
Medical Director, CLIMB Study, BWH
Director, Partners Pediatric MS Center, MGH

Disclosures

- **Consulting: Merck-Serono, Novartis, Biogen**
- **Clinical Trials Advisory Board: Novartis, Sanofi-Genzyme**
- **Industry Research funding: Merck-Serono, Novartis**
- **Non-industry: NIH, National MS Society, Guthy-Jackson Foundation, Peabody Foundation**

Overview

- Sex differences in MS risk
- Sex differences in MS course
- What can we learn about MS from changes during key sex-specific transitions?
- Effect of estrogen on parity and breast-feeding on MS risk and course (Riley Bove)
- Management of pregnancy in MS (Maria Houtchens)
- Menopause in MS: Effects and management (Riley Bove)

Sex-stratification in science and medicine

Sex versus gender:

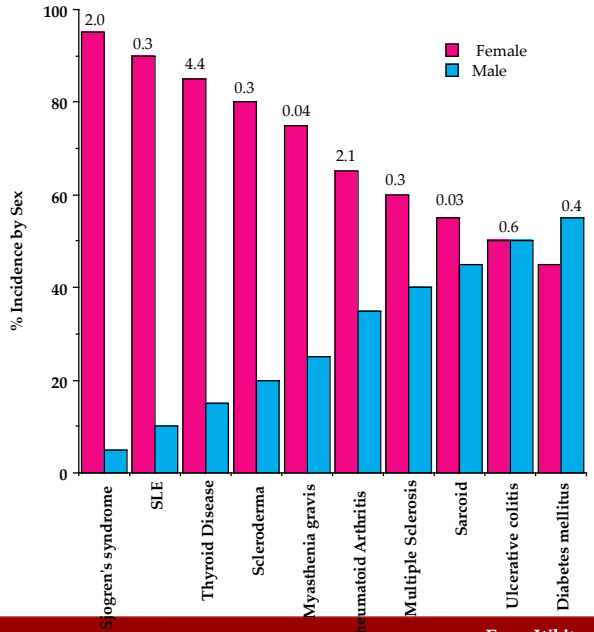
- Sex refers to biological processes
- Gender refers to behavioural processes

Sex-stratification in science and medicine

- **NIH has now mandated sex/gender-stratified studies – 2010 Strategic Plan for Women's Health:**

- Goal 1: Increase sex differences research in basic science studies.
- Goal 2: Incorporate findings of sex/gender in the design and development of new technologies, medical devices and therapeutic drugs.
- Goal 3: Actualize personalized prevention, diagnostics and therapeutics for girls and women.
  - Male and female animal models
  - Sex stratified analysis of human data - Sufficient power (numbers) to analyze male and female data
  - Supplemental funds to assist with sex-stratified analysis

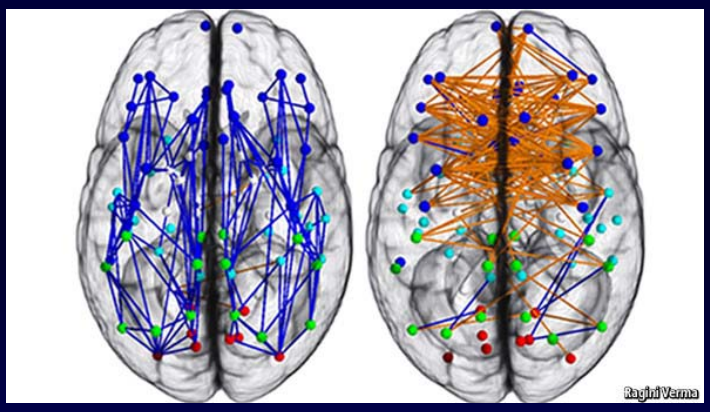
Sex Distribution in Common Autoimmune Diseases



From Whitacre; Nature Immunology, 2001

Sex-stratification in science and medicine

- Men and women are different!



Sex-stratification in science and medicine

Sex differences influence immune responses:

- Females generally have a lower burden of microbial infections than males
- Females have a higher prevalence of autoimmune diseases
- XX vs XY and X chromosome inactivation determine X-linked gene immune responses
- Fluctuations in estrogen levels influence B and T cell lineage commitment
- Estrogen effects on Th1 vs Th2 polarization affect pathogenesis of MS, RA and SLE

X-linked immune genes

a Receptors & associated proteins

AR	Androgen receptor
AGTR2	Angiotensin receptor 2
CSF2RA	Colony-stimulating factor 2 receptor α (granulocyte-macrophage)
GPCR	G-protein coupled receptors 23, 50, 101, 112, 119, 174 and CX-chemokine receptor 3
CYSLTR1	Cysteinyl leukotriene receptor 1
IL-1RAP1	Interleukin-1 (IL-1) receptor accessory protein-like 1
IL-1RAP2	IL-1 receptor accessory protein-like 2
IL-2RG	IL-2 receptor γ-chain
IL-3RA	IL-3 receptor α-chain
IL-9R	IL-9 receptor
IL-13RA1	IL-13 receptor α1-chain
IL-13RA2	IL-13 receptor α2-chain
IRAK	IL-1 receptor-associated kinase
NGFRAP1	Nerve-growth-factor receptor associated protein 1
TLR7	Toll-like receptor 7
TLR8	Toll-like receptor 8

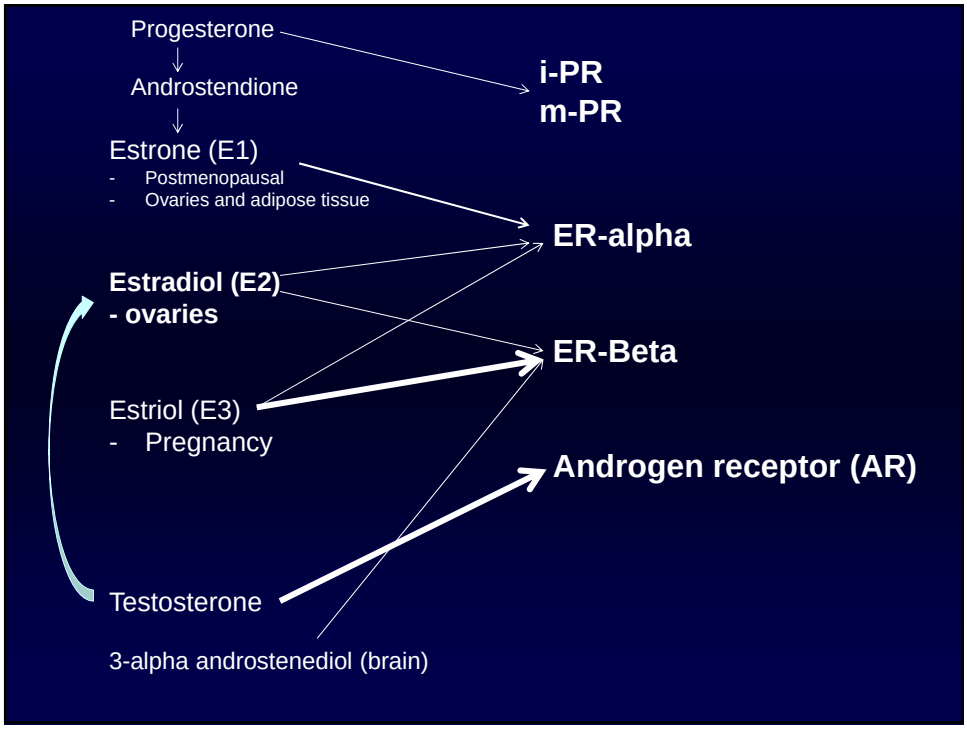
b Immune-response related proteins

XSCID	X-linked severe combined immunodeficiency
ELK1	Involved in B-cell development
EPAG	Early lymphoid activation protein
GATA1	GATA-binding protein 1
GTD	Gonadotropin deficiency
IDDMX	X-linked susceptibility to insulin-dependent diabetes
IGBP1	CD79A, immunoglobulin binding protein 1
IGSF1	Immunoglobulin superfamily member 1
ITGB1BP2	Integrin-β$_1$-binding protein 2
CD99	Also known as MIC2; associated with T-cell function
MTCP1	Mature T-cell proliferation 1
PFC	Properdin P factor, complement
TIMP1	Tissue inhibitor of metalloproteinase 1
CD40L	CD40 ligand
Z39IG	An immunoglobulin superfamily protein

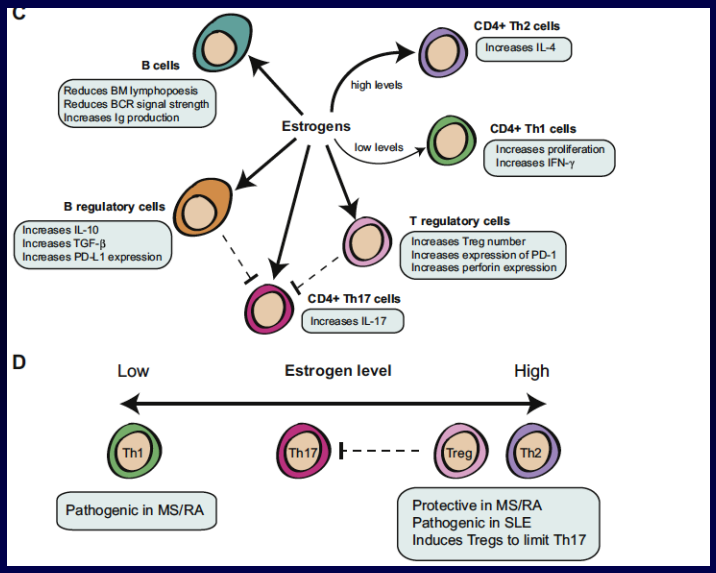
c Transcriptional & translational control effectors

RHOGAP	RAS homologue (RHO) GTPase activating proteins 4, 6
CDC42GEF	Cell-division cycle 42 guanine-nucleotide-exchange factors 6, 9
ETK	Also known as BMX
BTK	Bruton agammaglobulinaemia tyrosine kinase
CDX4	Caudal homeobox transcription factor 4
TRAP170	A co-factor for SP1 transcription factor activation
DUSP	Dual specificity phosphatases 9, 21
EEF	Eukaryotic translation elongation factors 1α13, β4
EIF	Eukaryotic translation initiation factor 1A*, 2a
FOXP3	Forkhead box P3 (associated with the development and function of regulatory T cells)
GAB3	Growth-factor-receptor-bound protein 2-associated binding protein 3
HDAC	Histone deacetylases 6, 8
IKKγ	IκB kinase; also known as NEMO
MAPKKK15	Mitogen-activated protein kinase kinase kinase 15
NFκBRF	Nuclear factor-κB (NF-κB) repressing factor
NRK	NF-κB-inducing kinase-related kinase
NXF	Nuclear RNA export factors 2, 3, 4, 5
PAK3	p21 (also known as CDKN1A)-activated kinase 3
PPP	Protein phosphatases 1, 2*, 6
PRKCI	Protein kinase Cι
S6K	Ribosomal protein S6 kinase
SWI/SNF	SWI/SNF-related, matrix associated, actin-dependent regulator of chromatin
STK9	Serine/threonine kinase 9
TAF1	TATA-box-binding protein-associated factor 1, TFIID subunit
UBE1	Ubiquitin-activating enzyme E1
UBE2A	Ubiquitin-conjugating enzyme E2A
USP	Ubiquitin-specific proteases 9*, 11, 26, 27, 511
WASP	Wiskott–Aldrich syndrome protein

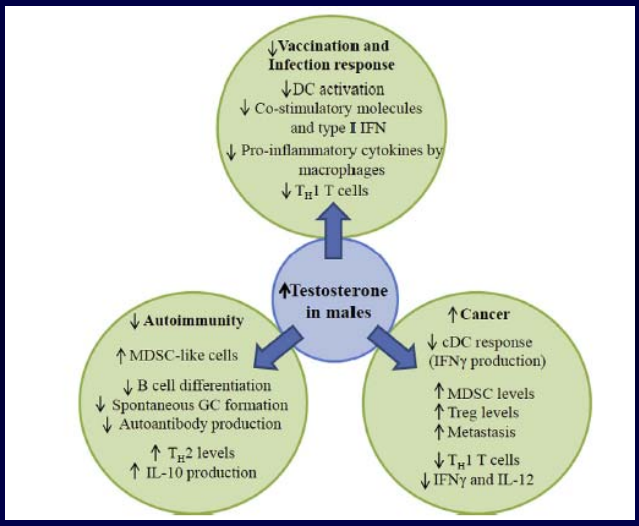
Nature Reviews | Immunology



Estrogen effects on immunity



Androgens –immune regulation



MS Risk and Sex

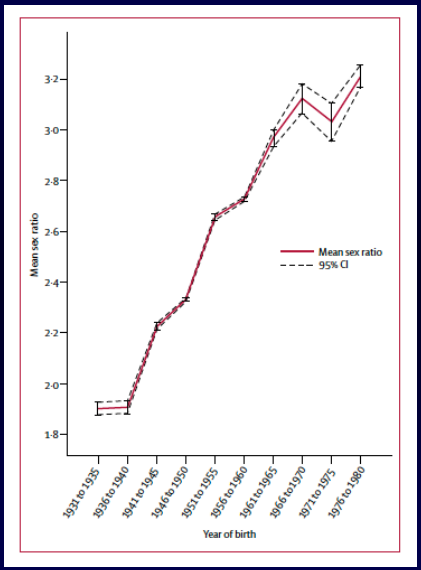
Genetics: Sex ratio in Multiple Sclerosis

- Mothers may be more likely to transmit the risk of MS, and of the HLA-DRB1*1501 risk allele, even when the mother is not affected.
- The HLA-DRB5*0101–HLA-DRB1*1501–HLA-DQA1*0102–HLA-DQB1*0602, extended haplotype is more common in F than in M patients, has a higher F:M ratio in MS subjects than in controls, and in families with two generations of MS, the F in the latest generation have an increased frequency of HLA-DRB1*15.
- Further analysis should stratify for sex (not control for sex)
- Exploration of X-linked immune genes and Y-protective genes

Bove and Chitnis, MSJ review 2013

Sex ratio in Multiple Sclerosis

- As with many autoimmune diseases, there is a female predominance in MS of approximately 3:1
- Evidence suggests that there is an increasing female prevalence of MS over the past 50 years, with a disproportionate increase in women



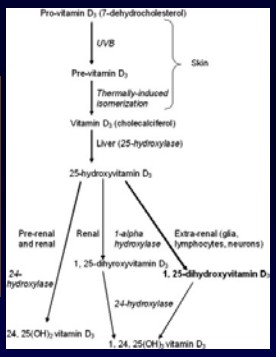
Orton, Lancet Neurology 2006

Environmental risk factors for MS – gender and sex effects

Risk factor	Gender influence	Sex influence
• Smoking	➉ ⇧females	• ?
• Vitamin D deficiency	• Sunlight/diet?	• Interaction with estrogen receptor
• Epstein-Barr virus exposure	• ?	• ?
• Obesity	➉ ⇧males and females	• ?
• Perinatal exposures?		

Vitamin D
The "Sunshine ~~Vitamin~~ Hormone"

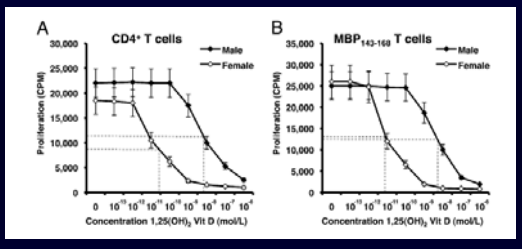
- 7-dehydrocholesterol is metabolized into Vitamin D3 by UVB skin exposure

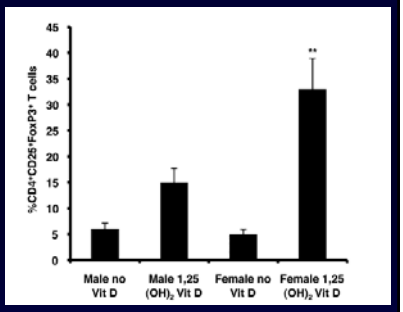


- Genetic mutations which affect Vitamin D synthesis and function

Mowry EM. J Neurol Sci 2011; 311:19-22.

Vitamin D effects on inhibition of CD4+ T cell proliferation and regulatory T cell formation is stronger in female mice





Correale, Journal of Immunology, 2010

Mechanisms of female immune "responsiveness" to Vitamin D

- Vitamin D degrading enzyme (CYP24A1) is decreased in females:males
- Estradiol mediates down-regulation of CYP24A1, and increases expression of Vitamin D receptor
- Addition of 17-B estradiol reproduces Vitamin D "female" effects in male T cells

Obesity in MS

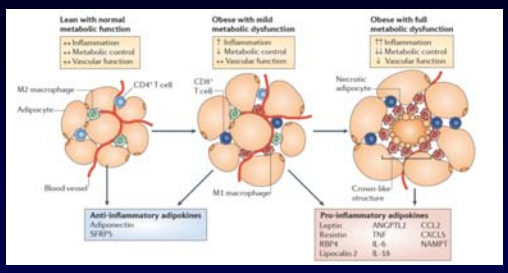
- Women who are obese (BMI>30kg/m2) at the age of 18 had a greater than two-fold increased risk of MS compared to those with normal BMI (Munger, Neurology 2009)
- Obese girls are twice as likely to develop MS as normal weight girls (Langer-Gould, Neurology 2013)
- Childhood obesity was associated with 2X risk of MS in females only (Munger, MSJ 2013)
- Obesity in women is associated with relapsing-onset MS (Marrie, Acta Scandivania 2011)
- Obesity in adolescence associated with MS risk in females (Kavak, MSJ 2014)

- Sex specific effects of obesity mechanisms?
- Sample size issues?

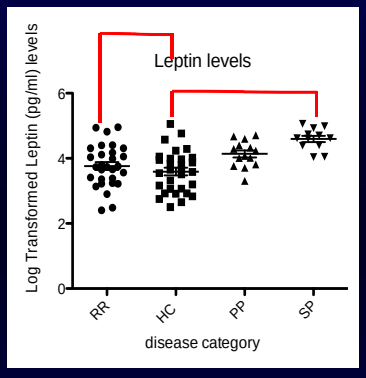
Adipose tissue and inflammation

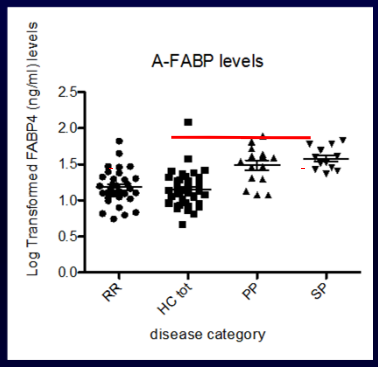
Obesity is characterized by a low-grade systemic inflammation due to the secretion of pro-inflammatory proteins in the blood.

Adipose tissue is an active secretory organ modulating appetite, energy expenditure, insulin sensitivity, endocrine and reproductive system, inflammation and immunity.



Increased leptin and A-FABP in RR and SPMS





- Controlling for age, leptin was increased in SPMS and RRMS compared to HC (RR compared to HC p<0.062, SP compared to HC p<0.001).
- Controlling for age and BMI, leptin is increased SPMS versus HC (p=0.007).
- Controlling for age and BMI, FABP was increased SPMS versus HC (p<0.007).

Messina, BMC Neurol 2013

Sex stratified, integrated hormones approach:

Significant effects of Vitamin D level and adiposity marker on disability and cognitive measures in women>men

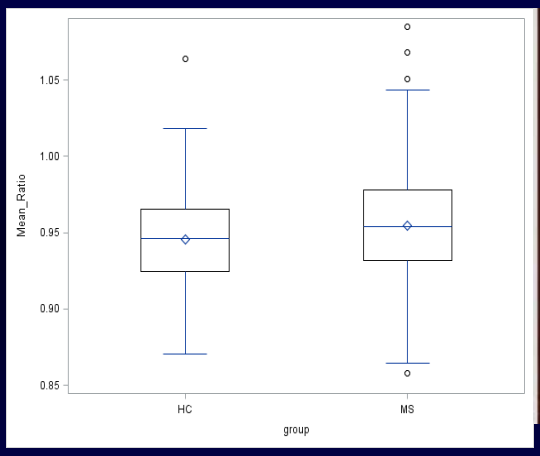
	UNIVARIATE ASSOCIATIONS			MULTIVARIATE ASSOCIATIONS		
	All subjects	Women	Men	All subjects	Women	Men
Cross-sectional EDSS (N=163) – Odds ratio greater than 1 indicates worse function						
Adiposity Markers						
Leptin	1.20 (0.060)	1.25 (0.072)	2.08 (0.079)	-	-	-
a-FABP	**1.26 (0.004)**	**1.27 (0.012)**	1.18 (0.44)	**1.27 (0.003)**	**1.31 (0.005)**	1.04 (0.86)
Additional markers						
25(OH)VitD	**0.69 (0.010)**	**0.69 (0.036)**	0.73 (0.20)	**0.67 (0.005)**	**0.64 (0.015)**	0.74 (0.22)
Testosterone	0.99 (0.55)	1.00 (0.97)	**0.95 (0.008)**	1.00 (0.71)	1.03 (0.79)	**0.95 (0.007)**
Cross-sectional SDMT (N=65) – positive score indicates better function						
Adiposity Markers						
Leptin	-0.03 (0.72)	-0.04 (0.71)	0.15 (0.75)	-	-	-
a-FABP	-0.14 (0.052)	-0.14 (0.12)	-0.15 (0.56)	-0.10 (0.17)	-0.12 (0.17)	-0.11 (0.66)
Additional markers						
25(OH)VitD	**0.32 (0.015)**	**0.40 (0.009)**	0.054 (0.87)	**0.30 (0.027)**	**0.36 (0.018)**	0.010 (0.98)
Testosterone	0.004 (0.53)	0.071 (0.60)	0.019 (0.22)	0.005 (0.51)	0.070 (0.57)	0.018 (0.26)

Bove, in preparation

Perinatal exposures and sex-stratified MS risk

- Breast-feeding is associated with a reduced risk of MS in males in Italian and Norwegian cohorts (Pugliatti, BMC Neurology 2015)
- Lower gestational Vitamin D is associated with increased risk of MS in women (Mirzaei, Ann Neurol 2011) – NURSES (FEMALE) STUDY
- 2D:4D ratio is associated with MS risk in males (Bove, Neurology 2015, in press) – FEMALES NOT STUDIED YET

2D:4D a proxy measure for prenatal androgen exposure is increased in males with MS vs. non-MS – reflecting lower prenatal androgen



Mean (SD) 2D:4D ratio was higher in MS (0.9546 (0.04)) than in non-MS subjects (0.9456 (0.03)) (p=0.038)

Bove et al, Neurology, in press

Effects of Hormonal transitions on MS

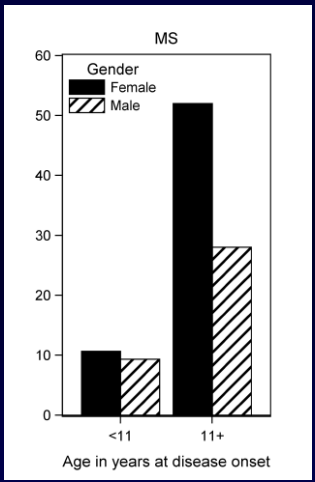
- **Pregnancy**
  - **Third trimester is protective for attacks in MS**
  - **Post-partum period - increased attacks**
- **Menopause**
  - **Some evidence of worsening disease course post-menopause**

Increased incidence and Female:Male ratio post-pubertally

- 150 pediatric MS patients from U.S. Network of Pediatric MS Centers
- Stratified by onset pre and post age 11

- 80% of pediatric MS cases occur after menarche (NPMSC)

- Puberty in females increases risk of MS in patients with first attack (Ahn, MSJ 2015)



Chitnis et al, AAN 2014

MS is worse in males

- Males have more rapid accrual of disability (Confavreux, Brain 2003)

Variable	No. of patients (n = 1844)	Time from onset of multiple sclerosis to assignment of a score of 4			Time from onset of multiple sclerosis to assignment of a score of 6			Time from onset of multiple sclerosis to assignment of a score of 7		
		Median (years)	95% CI	P value**	Median (years)	95% CI	P value**	Median (years)	95% CI	P value**
Gender										
Males	657	7.2	6.0–8.3	Reference	17.2	14.4–20.0	Reference	25.1	19.9–30.3	Reference
Females	1187	9.6	8.4–10.8	0.005	23.1	19.9–26.3	0.003	30.4	25.5–35.3	0.03

MS is worse in males

- Males are more likely to have a progressive-onset of MS (Raghavan, MSJ 2014)
  - PP group had a 1.09:1 male:female ratio Relapsing-onset ratio 1:2.89 male:female ratio
  - Higher mean age of onset (PP: 44.4±9.6; RO: 32.7±9.9; p < 0.0001)

Males have worse recovery from optic neuritis

- Younger age associated with better recovery

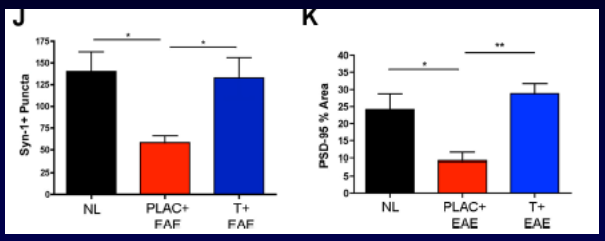
		AOMS	POMS	Unadjusted P-value	Adjusted^ P-value
Attack N (%)	*Mild (1)*	29 (11.5)	5 (13.16)	0.77	0.67
	Moderate (2)	101 (39.9)	13 (34.21)		
	Severe (3)	123 (48.6)	20 (52.63)		
Recovery N (%)	*Complete (1)*	202 (79.8)	36 (94.74)	**0.041**	**0.029**
	Fair (2)	23 (9.1)	1 (2.63)		
	Poor (3)	28 (11.1)	1 (2.63)		

- Males had worse recovery (adjusted OR=2.28, p=0.03)
- Subjects with more severe attacks had worse recovery (adjusted OR=5.24, p<0.001)

Malik, Neurology 2014

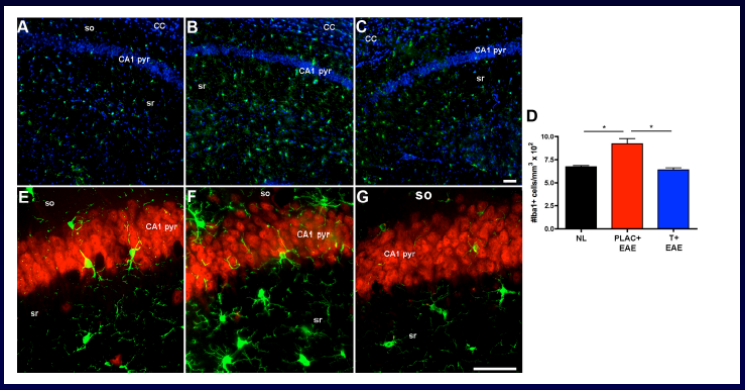
Testosterone has anti-inflammatory and neuroprotective effects in MS

- **EAE animal model: Increased inflammatory cell CNS infiltration in castrated male mice (Palaszynski, JNI 2004)**
- **Synaptic staining is preserved in testosterone-supplemented male mice with EAE (Ziehn, JN 2012)**



Testosterone has anti-inflammatory and neuroprotective effects in MS

Microglia are decreased in testosterone supplemented male mice with EAE



(Ziehn, JN 2012)

LOW TESTOSTERONE LEVELS IN MS MALES

Age group (years)	*N*	Testosterone, mean ng/dL (SD)	Subjects with testosterone < 288 ng/dL (%)
25–29	13	374 (168)	46
30–34	20	328 (99)	40
35–39	24	352 (130)	38
40–44	12	345 (108)	33
45–49	12	375 (125)	17
50–54	6	254 (159)	67
55+	9	308 (94)	44
Total	96	342 (126)	39

dL: deciliter; ng: nanogram; SD: standard deviation.

2.5th percentile: 348

1st percentile: 282

Framingham Heart Study Gen3 (456M, ages 18-40Y)
Bhasin et al, *JCEM* 2011(96)

Bove et al, *MS J* 2014

LOW TESTOSTERONE, WORSE DISEASE SEVERITY

	Hormone	Age adjusted	
		R	*P*
EDSS	T	– 0.21	**0.044**
	T/E2 ratio	– 0.26	**0.013**
	Free T	– 0.20	0.051

Legend: Odds ratio for a ten unit increase in each of the hormone measures (p-value).
Adjusted for age and disease duration and type

Bove et al, *MS J* 2014

LOW TESTOSTERONE, MORE COGNITIVE LOSS

	HORMONE	*N*	EST	95% CL	*P*-value
SDMT	Testosterone	40	0.63	0.14, 1.12	**0.012**
	T/E2 ratio	39	0.72	–0.27, 1.71	0.153
	Free T	40	34.3	7.8, 60.8	**0.011**

All of these analyses included an adjustment for age on the intercept.
GEE analysis for change in yearly change in EDSS or SDMT for a 100-unit increase in baseline hormones, controlling for age. A positive estimate reflects a lesser decline for the SDMT and reflects a greater decline for the EDSS.
CL: Confidence limits; EDSS: Expanded Disability Status Scale; EST: estimate; GEE: generalized estimating equation; SDMT: Symbol Digit Modalities Test; T: testosterone; T/E2: testosterone/estradiol

Age, disease duration and disease category-adjusted associations between hormone markers and change in disability level over time

Bove et al, *MS J* 2014

Use of testosterone as an MS therapy?

- 10 patient pilot study found beneficial effect on PASAT cognitive testing and brain atrophy measures (1 year study-crossover design)
  - Sicotte, Archives of Neurology 2007
- Potential for testosterone as an add-on therapy in males with MS
- NEURONEXT Study (BWH and UCLA)

TO TREAT OR NOT TO TREAT?

- Diagnosing hypogonadism
  - Levels *and* symptoms
- Potential benefits
  - Energy
  - Libido
  - Muscle mass
- Risks
  - CV
  - Prostate cancer
- Potential risks in MS men?
  - ?affect
  - ?disinhibition
  - ?less active, more CV risk?

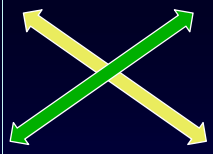
TAILORING MS THERAPIES – PERSONALIZED MEDICINE

MODIFIED BY AGE, SEX, ENVIRONMENTAL FACTORS, GENETICS

OPTIMIZE BY AGE, SEX, ENVIRONMENTAL FACTORS, GENETICS

MS MECHANISMS

- T Cell activation
- APC activation
- Epitope spreading
- B cell stimulation
- Antibody production
- Cortical lesions and gray matter pathology
- Dying back axonopathy
- Diffuse activation of glial cells – microglia and astrocytes
- Mitochondrial dysfunction and oxidative stress
- Presence of meningeal follicles
- Impaired CNS repair processes



MECHANISMS MS THERAPIES

- B-IFN
- Increase Th2 response
- Decrease Th1/Th17 responses
- Enhance Tregs
- T, B cell cytostatics
- Modify APC function
- Prevent immune cell migration/BBB penetration
- Stabilize mitochondria
- Modify microglial, astrocyte function
- Enhance neuroprotection
- Enhance remyelination

Sex differences in conventional MS DMT effects?

- **(NO) Gender effects on intramuscular interferon beta-1a in relapsing-remitting multiple sclerosis: analysis of 1406 patients (Rudick, Multiple Sclerosis, 2011 Mar; 17(3):353)**
- Outcome measures included time to first relapse, annualized relapse rate, time to disability progression, number of gadolinium-enhanced lesions, adverse events, laboratory evaluations, and neutralizing antibodies.

Have we looked hard enough for sex differences in response to conventional DMT?

Sex differences in conventional MS DMT effects?

- **Glatiramer acetate in PPMS – PROMISE Trial (Wolinsky, Ann Neurol 2007)**
  - **Post hoc analysis suggested an effect in males**
    - male patients diverged early from Placebo-treated male subjects (hazard ratio, 0.71 [95% confidence interval, 0.53-0.95]; p = 0.0193).
- **Further work concluded no treatment by gender interaction in PPMS and ROMS with GA (Wolinsky, J. Neurol. Sci 2009)**

Summary

- Increasing female predominance in MS
- MS course differs in males and females
- 2D:4D ratio is increased in men with MS (reflecting lower in utero androgens)
- Low testosterone may be associated with increased disability in men with MS
- Unclear if sex differences in response to conventional MS DMTs – more research required….

Take home messages

- For the clinician:
  - Consider sex-specific disease course in managing and treating patients
  - Consider role of adjunct hormonal therapies
- For the researcher:
  - Consider studies that include sufficient numbers of both sexes
  - Exploration of sex-specific disease mechanisms
- For the pharmacologist:
  - Consider sex-stratified analysis of treatment studies with sufficient numbers
  - Exploration of sex-specific treatment mechanisms and adjunct therapies

Overview

- Sex differences in MS risk
- Sex differences in MS course
- What can we learn about MS from changes during key sex-specific transitions?
- Effect of estrogen on parity and breast-feeding on MS risk and course (Riley Bove)
- Management of pregnancy in MS (Maria Houtchens)
- Menopause in MS: Effects and management (Riley Bove)